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Report 670

Effects of available phosphorus (aP), calcium/aP ratio, and growth rate on P deposition, P digestibility, performance and leg quality in broilers.

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Abstract
The standard Ca/aP ratio (2.2) can be recommended for application in practical diets. Based on the results of this experiment, no proof was found for our hypothesis that the development of the skeleton in fast growing broilers could not keep pace with the gain of the soft tissues. This experiment showed that the P requirement was not fulfilled with the low aP level in the diet. For determination of the optimal dietary aP level, however, a dose – response experiment should be performed.

Keywords
Available phosphorus (aP), Ca/aP ratio, growth rate, tibia, digestibility, performance

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Preface

On request of the Dutch Ministry of Economic Affairs, as well as of the Dutch Product Board Animal Feed, this study was performed to investigate the effects of available phosphorus (aP), calcium/aP ratio, and growth rate on P deposition, P digestibility, performance and leg quality in broilers. A working group (Werkgroep P-Mineraal), consisting of representatives of the funding organisations, farmers and feed, producers guided this study. We would like to thank the members of this working group for their valuable input to all phases of this project.

Marinus van Krimpen
Project leader

Samenvatting

De fosfaatexcretie van vleeskuikens wordt berekend op basis van de fosfor (P) -opname via het voer en de P-vastlegging in het dier. Op dit moment wordt voor een slachtrijp vleeskuiken een forfaitair P-gehalte van 4,4 g/kg gehanteerd. Dit getal is gebaseerd op een inmiddels 12 jaar oude studie (Versteegh en Jongbloed, 2000). Echter, sindsdien is de groeisnelheid van vleeskuikens al weer aanzienlijk toegenomen. De kuikens uit die studie bereikten op 42 dagen een gewicht van 2072 g, terwijl een modern kuiken op deze leeftijd momenteel 2768 g weegt (Ross 308 manual). Door veranderingen in groeicapaciteit, percentage borstfilet (lichaamssamenstelling) en voersamenstelling kunnen verschuivingen in de vlees-bot verhouding (meer vlees – minder bot) en het P-gehalte in het dier zijn opgetreden. Op dit moment is onduidelijk of als gevolg van deze verschuiving de behoefte aan opneembaar fosfor (oP) per kg voer is toe- of afgenomen. Met name het P-gehalte in het karkas heeft een grote invloed op de berekende oP-behoefte. Daarom is het noodzakelijk de vastlegging van fosfor bij vleeskuikens te actualiseren, waarbij we specifiek inzicht willen krijgen in de verdeling van de P-retentie over skelet en vlees.
Moderne snelgroeiende vleeskuikens groeien zo snel dat zij mogelijk onvoldoende in staat zijn om de skeletopbouw gelijk op te laten lopen met de groei van de rest van het lichaam (m.n. spiergroei). Als deze veronderstelling correct is, zouden kuikens met een tragere groei relatief meer P vastleggen in het skelet dan snelgroeiende kuikens. Onvoldoende P-vastlegging in het skelet geeft een verhoogd risico op beengebreken. Verwacht wordt dat verhoging van het oP-gehalte in het voer boven de huidige berekende oP-behoefte resulteert in meer P-vastlegging in het skelet en verhoging van de pootkwaliteit.
De P-benutting kan ongunstig beïnvloed worden door een hoge Ca/oP-verhouding in het voer, omdat in het maagdarmkanaal P en Ca samen complexen vormen die voor het kuiken niet meer benutbaar zijn. Bij een lage Ca/oP-verhouding kan de Ca-voorziening een beperkende factor worden voor een goede botaanzet. We veronderstellen dat bij een marginale P-voorziening een relatief lage Ca/oP-verhouding resulteert in een lagere skeletaanzet, waardoor meer oP beschikbaar blijft en gebruikt wordt voor vleesaanzet. Dit zou echter ten koste kunnen gaan van de skelet (bot) kwaliteit.

Doelstelling
Het vaststellen van de hoofdeffecten van twee oP-niveaus en drie Ca/oP-verhoudingen in het voer, twee groeisnelheden van vleeskuikens, en hun onderlinge interacties op de P-verteerbaarheid, de aanzet van P, Ca en N, de verdeling van P en Ca over het skelet en het vlees, de dierprestaties en de beenwerkkwaliteit, met als uiteindelijke doel via voedingsmaatregelen de P-excretie te verlagen zonder de pootkwaliteit en dierprestaties nadelig te beïnvloeden.

Materiaal en methode
De proef kende 12 proefbehandelingen, zoals in Tabel S1 weergegeven.

Tabel S1. Overzicht van proefbehandelingen.

Behandeling	oP-niveau	Ca/oP-ratio	Groeisnelheid
1	Laag	Laag	Hoog
2	Laag	Gangbaar	Hoog
3	Laag	Hoog	Hoog
4	Laag	Laag	Laag
5	Laag	Gangbaar	Laag
6	Laag	Hoog	Laag
7	Hoog	Laag	Hoog
8	Hoog	Gangbaar	Hoog
9	Hoog	Hoog	Hoog
10	Hoog	Laag	Laag
11	Hoog	Gangbaar	Laag
12	Hoog	Hoog	Laag

Bij het lage oP-niveau kregen de kuikens voer met een oP-gehalte dat 20% lager was dan het huidige advies (CVB, 2010), terwijl het oP-gehalte bij het hoge oP-niveau 20% boven het huidige advies lag (Tabel S2). Het voer met de lage Ca/oP-verhouding had een ratio van 1,5, de gangbare Ca/oP-verhouding had een ratio van 2,2 en de hoge Ca/oP-verhouding had een ratio van 2,9. De verschillen in Ca/oP-ratio werden gerealiseerd door te variëren met het aandeel krijt in het voer. Om de lage

groeisnelheid te realiseren, kreeg deze categorie kuikens een voer met lagere gehalten aan verteerbare aminozuren. Daarnaast werden ze zodanig beperkt gevoerd dat de uiteindelijke groeisnelheid 20% lager was dan de dieren met de hoge groeisnelheid.

Tabel S2. oP-niveau van de voeders per gewichtstraject.

Traject, d [1)]	Doelgewicht (g)	oP, g/kg		
		CVB-advies	CVB-20%	CVB+20%
0-10		4.0	4.0	4.0
10-35	1750	3.1	2.5	3.7
35-42	2300	2.8	2.4	3.4

[1)] De traaggroeiende kuikens bereiken het eindgewicht van 2300 g ca. 1 week later.

Per behandeling werden 4 herhalingen (= grondhokken) ingezet, zodat er in totaal 48 grondhokken bij dit experiment zijn betrokken. Voor het vaststellen van de verteerbaarheid is een deel van de kuikens op een gewicht van ca. 1600 gram overgeplaatst naar balanskooien, waarin faeces werd opgevangen.

<u>Resultaten</u>

P-gehalte van vleeskuikens bij ca. 1750 g
Het P-gehalte van vleeskuikens is afhankelijk van een groot aantal factoren. Het P-gehalte in het complete dier steeg bij het hogere oP-niveau van het voer met 0,4 g/kg van 4,4 naar 4,8 g/kg EBW (empty body weight). Daarnaast was ook de Ca/oP-verhouding in het voer hierop van invloed. Bij een hoge en gangbare Ca/oP-verhouding was het P-gehalte in het dier hoger dan bij een lage Ca/-oP verhouding (4,7 vs. 4,3 g/kg EBW). Ook de sekse was hierop van invloed. Bij haantjes was het P-gehalte 4,7 g/kg, terwijl dit bij hennetjes 4,5 g/kg EBW bedroeg.
In tegenstelling tot onze verwachting had de groeisnelheid van de kuikens geen effect op het P-gehalte van het skelet. Dit betekent dat de snelle groei van vleeskuikens geen belemmering vormt voor het aanzetten van Ca en P in de botmatrix. Uiteindelijk was het P-gehalte van de langzame groeiers wel numeriek, maar niet significant (P=0,11) hoger dan van snelle groeiers (4,68 vs. 4,55 g/kg EBW). Het iets hogere P-gehalte bij de langzame groeiers was het gevolg van een aantoonbaar hoger aandeel skelet (8,2 vs. 7,1%) en een lager aandeel weke delen in de kuikens.

Botsterkte
Ondanks de grote verschillen in oP-gehalte van het voer had dit geen effect op de botsterkte. Wel bleek dat haantjes sterkere botten hadden dan hennetjes. Ook bleken snelle groeiers sterkere botten te hebben dan langzame groeiers. De botsterkte nam bovendien toe naarmate de Ca/oP-verhouding toenam. De verschillen in botsterkte hadden overigens geen enkel effect op de loopeigenschappen van de kuikens.

Dierprestaties
Kuikens die het voer met het lage oP-gehalte kregen groeiden gemiddeld 2,8 g/d minder snel dan de kuikens die het voer met het hoge oP-gehalte kregen. Tijdens de eerste 24 dagen van het groeitraject groeiden de kuikens ca. 2 g/d minder snel als ze het voer met de lage Ca/oP-verhouding kregen, terwijl er geen verschil was in groeisnelheid tussen de kuikens die het voer met de gangbare of hoge Ca/oP-verhouding kregen. De Ca/oP-verhouding in het voer had geen effect op de voederconversie. De verschillen in groeisnelheid zijn dus volledig toe te schrijven aan verschillen in voeropname.

Fecale P-benutting
De fecale P-benutting, berekend als de hoeveelheid P die niet is uitgescheiden gedeeld door de totale hoeveelheid opgenomen P *100%, hing in sterke mate af van het oP-niveau van het voer. Gemiddeld was de P-benutting bij het lage oP-niveau 41,4% tegenover 31,1% bij het hoge oP-niveau. De Ca/oP-verhouding had hier echter wel invloed op. Bij het lage oP-niveau was de P-benutting het hoogst bij de kuikens met de hoogste Ca/oP-verhouding in het voer (44,2%), terwijl deze bij de kuikens met het hoge oP-niveau het hoogst was de kuikens die de lage (32,9%) of gangbare (31,6%) Ca/oP-verhouding kregen.

Fosfaatuitscheiding
Het oP-niveau in het voer had een groot effect op de fosfaatuitscheiding in de mest. Bij het lage oP-niveau was de fosfaatuitscheiding 35% lager dan bij het hoge oP-niveau (0,79 vs. 1,20 g/d). Bij het lage oP-niveau in het voer had de Ca/oP-verhouding geen effect op de fosfaatuitscheiding, terwijl de fosfaatuitscheiding bij het hoge oP-niveau steeg van 1,16 naar 1,26 g/d naarmate de Ca/oP-verhouding hoger werd (Tabel 3). Een combinatie van een hoog oP-niveau en een hoge Ca/oP-verhouding kan resulteren in de vorming van slecht verteerbare complexen van calcium met fosfaat.

Tabel S3. Effect van oP-niveau en Ca/oP-verhouding op de fosfaatuitscheiding (g/d/d).

	Laag oP-niveau	Hoog oP-niveau
Lage Ca/oP verhouding	0,79	1,16
Gangbare Ca/oP verhouding	0,79	1,19
Hoge Ca/oP verhouding	0,78	1,26
Gemiddeld	0,79	1,20

Standaardafwijking = 0,018

<u>Conclusies</u>

- Het oP-gehalte van het voer en de Ca/oP-verhouding hebben geen invloed op de hoeveelheid as, Ca en P in de weke delen van het kuiken. Langzaam groeiende kuikens hebben een lager P en N gehalte in de weke delen dan snel groeiende kuikens.
- Gemiddeld over alle behandelingen heen wordt 75,3% van het in het lichaam aanwezige Ca en 53,6% van het in het lichaam aanwezige P opgeslagen in de botten.
- Gemiddeld over alle behandelingen bevat het lege lichaam 4,6 g/kg P en 29,7 g/kg N.
- Kuikens die voer met een hoog oP-gehalte (20% boven het CVB-advies) krijgen, hebben relatief meer bot met in het bot een 8% hoger P-gehalte en leggen daardoor 8% meer P vast in het lichaam in vergelijking met kuikens die voer met een laag oP-gehalte krijgen (20% onder het CVB advies). Het verstrekken van het voer met een hoog oP-gehalte zorgt voor een snellere groei (5%) en betere voederconversie (4%). Verstrekking van voer met een hoog oP-gehalte leidt echter tot een verlaging van de ileale Ca- en P-verteerbaarheid en tot 35% meer fosfaatexcretie via de mest.
- Een lage Ca/oP-verhouding (1,5) remt de groei van de kuikens aan het begin van het groeitraject en verlaagt het P-gehalte in het lege lichaam ten opzichte van een gangbare (2,2) of hoge (2,9) Ca/oP-verhouding. Bij kuikens die voer met een hoog oP-gehalte krijgen, resulteert een hoge Ca/oP-verhouding in het voer in meer fosfaatuitscheiding via de mest in vergelijking met de gangbare of lage Ca/oP-verhouding. Het hanteren van de gangbare Ca/oP-verhouding geeft het hoogste N-gehalte in weke delen en in het lege lichaam, het hoogste relatieve skeletaandeel en Ca- en P-gehalte in het skelet, het hoogste relatieve tibia gewicht en het hoogste lichaamsgewicht op dag 35.
- Het P-gehalte in het skelet van langzaam groeiende vleeskuikens wijkt niet af van dat van snel groeiende kuikens. Wel is het relatieve botaandeel bij langzaam groeiende kuikens 15% hoger dan bij snel groeiende kuikens waardoor bij deze kuikens relatief een hoger aandeel van alle Ca en P in het lichaam is opgeslagen in de botten. De tibia van langzaam groeiende kuikens bevat meer Ca en P, maar deze tibiae waren minder sterk dan van de kuikens met de gangbare groei.

<u>Aanbevelingen:</u>
Het verstrekken van voer met een lage Ca/oP-verhouding (1,5) heeft een negatieve invloed op de dierprestaties. De Ca- en P-verteerbaarheid neemt af naarmate de Ca/oP-verhouding toeneemt. Het hanteren van de gangbare Ca/oP-verhouding (2,2) resulteert in een vergelijkbare of betere ontwikkeling van het skelet en tibia in vergelijking met de hoge Ca/oP-verhouding (2,9). Daarom wordt voor vleeskuikenvoer de gangbare Ca/oP-verhouding aanbevolen.
Dit experiment heeft onze hypothese, namelijk dat snel groeiende kuikens moeite hebben om de skeletontwikkeling gelijk op te laten lopen met die van de rest van het lichaam, niet bevestigd.
Op basis van de modelmatige (factoriële) benadering, zoals deze in Nederland gehanteerd wordt, zou het oP-gehalte in startvoeders (0 – 10 d) 5,5 g/kg, in groeivoeders (10 – 30 d) 3,3 g/kg en in eindvoeders (> 30 d) 2,9 g/kg moeten bedragen. Deze aanbevelingen liggen respectievelijk 37%, 7% en 4% hoger dan de huidige Nederlandse CVB-normen.
Nieuw dierexperimenteel onderzoek moet uitwijzen of de uitkomsten van deze modelmatige benadering overeenkomt met de bevindingen gemeten aan de dieren zelf. Deze proef toont aan dat de P-behoefte van vleeskuikens niet volledig gedekt wordt bij een oP-gehalte in het voer dat 20% onder het CVB-advies ligt. In dit experiment zijn echter slechts twee oP-niveaus onderzocht, zodat

hieruit niet de oP-behoefte van het huidige moderne vleeskuiken afgeleid worden. Voor het vaststellen van de oP-behoefte is een dosis-respons experiment met meerdere oP-niveaus noodzakelijk. Dit aanvullende onderzoek wordt in 2013/2014 uitgevoerd.

Summary

The phosphate excretion of broilers will be calculated by subtracting the phosphorus (P) deposition in the bird from the P intake by feed. Currently, the agreed P-content of a broiler at slaughter age in the Netherlands amounts 4.4 g/kg. This value is based on a study published in 2000 (Versteegh en Jongbloed, 2000). Since that time, however, the growth potential of broilers has been seriously increased. Broilers in that study had a body weight of 2072 g at 42 days of age, whereas nowadays broilers at the same age weigh 2768 g (Ross 308 manual). Due to changes in growth potential, body composition (percentage of breast meat) and diet composition, the ratio between soft tissues and bones (more soft tissues – less bones) might be changed, which consequently may affect the P-content of a broiler. It is unclear, however, whether these changes resulted in an increase or decrease of the available P (aP) requirement. In the calculation of the aP-requirement, by use of the factorial approach, the P-content of the body is a main factor. Therefore, it is necessary to update the P-deposition in broilers. To understand the mechanism behind, it is also relevant to obtain knowledge regarding the distribution of P-deposition over the skeleton and the soft tissues.
It was hypothesized that the growth rate of modern fast growing broilers is so high that the development of the skeleton is not able to keep pace with the development of the rest of the body (mainly muscle growth). In that case, slow growing broilers should deposit relatively more P in the skeleton compared to fast growing birds. Insufficient P-deposition in the skeleton increases the risk of leg problems. Moreover, it is expected that an increase of the dietary aP-supply above the current aP-requirement will result in an increased P-deposition in the skeleton and an increased leg quality of the birds.
Results from literature showed that a high dietary Ca/aP ratio negatively affected the P-utilisation, because of the formation of Ca-P complexes in the GIT. The Ca and P that is bound to these complexes is not available for the birds. At a low Ca/aP ratio, however, the Ca-supply might become the limiting factor for bone formation. We hypothesize that the condition of marginal P-supply combined with a low Ca/aP ratio results in retarded bone formation. As a consequence, relatively more aP remains available for meat deposition. This condition, however, could hamper skeleton (bone) quality.

<u>Aim</u>
To investigate the main effects of two dietary aP-levels, three dietary Ca/aP ratios, and two growth rates of the broilers, as well as their interactions, on P-availability, the P, Ca and N deposition in the bird, the distribution of P and Ca over skeleton and soft tissues, performance and leg quality, with the final aim to reduce P-excretion by nutritional strategies without negatively affecting animal performance and leg quality.

<u>Material and methods</u>
This experiment comprised 12 dietary treatments, as shown in Table S1.

Table S1. Overview of dietary treatments.

Treatment	aP-level	Ca/aP-ratio	Growth rate
1	Low	Low	High
2	Low	Standard	High
3	Low	High	High
4	Low	Low	Low
5	Low	Standard	Low
6	Low	High	Low
7	High	Low	High
8	High	Standard	High
9	High	High	High
10	High	Low	Low
11	High	Standard	Low
12	High	High	Low

The low aP diet had a 20% reduced aP-level compared to the currently recommended level (CVB, 2010), whereas the high aP diet had a 20% increased aP-level (Table S2). The Ca/aP ratio of the diets with the low, standard and high Ca/aP ratio were 1.5, 2.2 and 2.9, respectively. Differences in Ca/aP ratio were realized by varying the amount of limestone in the diets. We aimed to reduce the

growth rate of the slow growing birds by 20% compared to the fast growing birds. Therefore, these birds received a diet with reduced contents of digestible amino acids. Moreover, restricted feeding was applied.

Table S2. aP-level of the diets per period.

Period, d [1)]	Target weight (g)	aP, g/kg		
		CVB-recom.	CVB-20%	CVB+20%
0-10		4.0	4.0	4.0
10-35	1750	3.1	2.5	3.7
35-42	2300	2.8	2.4	3.4

[1)] The slow growing birds needed one week more to reach the final target weight (2300 g).

Each treatment was replicated four times (= four floor pens). Therefore, this experiment comprised 48 floor pens in total. When the birds weighed about 1600 g, part of the birds were moved to digestibility cages for faeces collection.

Results

P-content of broilers at about a body weight of 1750 g
The P-content of broilers is affected by the dietary aP-content, the Ca/aP ratio and the gender of the birds. The P-content of the birds fed the high aP diet increased by 0.4 g/kg from 4.4 to 4.8 g/kg EBW (empty body weight). The P-content of birds that were fed a diet with a standard or high Ca/aP ratio was increased compared to birds that were fed the diet with the low Ca/aP ratio (4.7 vs. 4.3 g/kg EBW). Male birds had a 0.2 g/kg higher P-content compared to female birds (4.7 vs. 4.5 g/kg EBW). The P-content of the slow growing birds was numerically higher compared to the fast growers (4.68 vs. 4.55 g/kg EBW), although this difference was not significantly different (P=0.11). This slightly increased P-content was the results of a higher share of skeleton (8.2% vs. 7.1%) and a lower share on soft tissues in the body.

Bone strength
Despite the large differences between treatments, dietary aP-content did not affect bone strength. Male broilers had stronger bones compared to females. Fast growing birds also had stronger bones compared to slow grower. The bone strength increased with increasing Ca/aP ratio. Bone strength, however, showed no relationship with the gait score of the birds.

Performance
The growth rate of broilers that were fed the low aP diet was on average reduced by 2.8 g/d compared to broilers fed the high aP diet. In the period of d1 – d24, growth rate of birds that were fed the diet with the low Ca/aP ratio was reduced by 2.0 g/d, compared to birds fed diets with the standard of high Ca/aP ratio. The Ca/aP ratio of the diet did not affect feed conversion ratio, indicating that differences in growth rate could be fully attributed to differences in feed intake.

Faecal apparent P-digestibility
The faecal apparent P-digestibility, calculated by the ratio of not-excreted amount of P/ total P intake * 100%, was highly affected by the dietary aP content. P-digestibility amounted 41.4% if the low aP diet was supplied versus 31.1% in case of the high aP-diet. Interaction effects of aP content and Ca/aP ratio, however, were observed. In case of the low aP diet, P-digestibility was highest in birds fed the diet with the high Ca/aP ratio (44.2%), whereas in case of the high aP diet, P-digestibility was highest in birds fed the diet with the low (32.9%) or the standard (31.6%) Ca/aP ratio.

Phosphate excretion
The dietary aP-level largely affected faecal phosphate excretion. Feeding the low aP-diet resulted in 35% decreased phosphate excretion, compared to feeding the high aP-diet (0.79 vs. 1.20 g/d). In case of feeding the low aP-diet, the Ca/aP ratio did not affect phosphate excretion, whereas in case of feeding the high aP-diet the phosphate excretion increased with increasing the Ca/aP ratio (from 1.16 to 1.26 g/d), as shown in Table S3. Combining a high aP level in the diet with a high Ca/aP ratio might result in the formation of badly absorbable calcium-phosphate complexes.

Table S3. Effect of dietary aP-level and Ca/aP-ratio on the phosphate excretion (g/d).

	Low aP-level	High aP-level
Lowe Ca/aP ratio	0.79	1.16
Standard Ca/aP ratio	0.79	1.19
High Ca/aP ratio	0.78	1.26
Average	0.79	1.20

Standard deviation = 0.018

Conclusions

- The contents of ash, Ca and P in soft tissues were not affected by aP level and Ca/aP ratio. The P and N content in soft tissues of slow growing birds were reduced compared to standard growing birds.
- Among treatments, 75.3% of body Ca and 53.6% of body P was deposited in the skeleton.
- Among treatments, P and N contents in empty body weight amounted 4.6 and 29.7 g/kg, respectively.
- Broilers that are fed a high dietary aP level (20% higher than recommended) had a 8% higher P content in empty body weight, because of an increased skeleton portion and an 8% elevated P content in the skeleton, compared to broilers fed a low dietary aP level (20% below the recommended level). Feeding the diet with the high aP level resulted in improved BWG (5%) and FCR (4%). Feeding a high dietary aP level reduced ileal Ca and P digestibility, whereas faecal phosphate excretion increased by 53%.
- A low Ca/aP ratio (1.5) in the diet reduced BWG in the starter and grower period, and the P content in empty body weight, compared to a standard (2.2) or high (2.9) Ca/aP ratio. In birds fed a high dietary aP level, a high Ca/aP ratio resulted in an increased phosphate excretion compared to birds fed the standard or low Ca/aP ratio. The ash content in the skeleton, and the tibia strength increased with increasing Ca/aP ratio, whereas the ileal Ca and P digestibility linearly decreased with an increasing Ca/aP ratio. Applying the standard Ca/aP ratio resulted in the highest N content in soft tissue and in empty body weight, in the highest skeleton portion and Ca and P content in the skeleton, in the highest (relative) tibia weight, and in the highest BW at 35 d of age.
- The P content in slow growing birds did not significantly differ from that of fast growing birds. The skeleton portion in empty body weight was increased by 15% in slow growers compared to fast growers, consequently resulting in an elevated Ca and P portion in the skeleton. The tibia of slow growers contained more Ca and P, but the tibia strength was reduced compared to the fast growers.

Recommendations

Feeding a diet with a low Ca/aP ratio (1.5) negatively affected broiler performance, whereas the Ca and P digestibility reduced with increasing dietary Ca/aP ratios. In line with our hypothesis, it was observed that feeding a diet with the standard Ca/aP ratio (2.2) resulted in a similar or better skeleton and tibia development compared to the high Ca/aP ratio (2.9). Therefore, the standard Ca/aP ratio can be recommended for application in practical diets. Based on the results of this experiment, no proof was found for our hypothesis that the development of the skeleton in fast growing broilers could not keep pace with the gain of the soft tissues.

According to the factorial approach system that is common in the Netherlands, starter diets (0 – 10 d of age) should contain an aP level of 5.5 g/kg, grower diets (10 – 30 d of age) 3.3 g/kg and finisher diets (> 30 d of age) 2.9 g/kg. These requirements are 37%, 7% and 4% higher compared to the current Dutch CVB recommendations.

A new animal experiment has to be performed to determine whether the results of the factorial approach are in line with the findings measured in the birds itself. This experiment showed that the P requirement was not fulfilled with the low aP level in the diet. For determination of the optimal dietary aP level, however, a dose – response experiment should be performed. This additional study will be carried out in 2013/2014.

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1 Introduction

Broilers require phosphorus (P) for muscle and bone growth and a number of metabolic processes. A marginal P supply was shown to reduce bone development and broiler performance (Waldenstedt, 2006; Abudabos, 2012). However, avoiding an unnecessary high P content of the diet is becoming increasingly important because of the environmental pressure, e.g. eutrophication, and increasing expenses because P is a non-renewable resource (Selle and Ravindran, 2007). The portion of P that exceeds the requirement will be excreted in faeces and P excretion is closely related to the P intake (Abudabos, 2012). Therefore, broilers must be fed as close as possible to their nutritional P requirements.

Based on a literature review, dose – response relationships between the dietary non phytate phosphorus (nPP) content and feed intake, BWG, FCR and tibia ash contents were reported (Letourneau-Montminy et al., 2010). Several authors have shown that for realizing maximum bone ash a higher P supply was required compared to the P requirement for maximal growth and feed efficiency (Driver et al., 2005; Abudabos, 2012). In the past, maximum bone ash was considered to be the criterion for determining the P requirement of broilers (Waldroup et al., 1975). Against the background of environmental regulations, scarcity of P-resources, it is very doubtful whether maximum bone ash should be basis for determining P requirement.

The P requirement of broilers can also be estimated by using a factorial approach, in which the requirement depends on the P need for maintenance, the amount of P retained in the body and on the P utilisation (WPSA, 1985). In this approach, P retention in the body is a very determining factor. Data regarding P retention in the body of broilers, however, are scarcely available. The Dutch factorial approach, for instance, most recently described by Jongbloed and Kemme (2005) is based on P retention values published in 2000 (Versteegh and Jongbloed, 2000). Although more recent data regarding P deposition in broilers became available (Dieckmann, 2004), this information is not very up to date in light of the rapid changes in growth potential of broilers as a consequence of the continuous selection for growth (Havenstein et al., 2003). It is expected that the ratio between the skeleton and soft tissues has been changed in contemporary broilers compared to broiler strains of one or two decades ago, which consequently affects the N, Ca and P content in broiler carcasses. Therefore, more insight in total mineral content and partitioning over different body segments in modern broilers is required.

Moreover, P utilisation is affected by many other factors, such as P availability and Ca/available P (aP) ratio. Although Ca is essential for the deposition of P in the bones, an oversupply of Ca has a negative effect on P digestion (Dieckmann, 2004; Delezie et al., 2012). It was hypothesized that particular a high Ca/aP ratio combined with a marginal aP level would be harmful for bone development of broilers. A too low Ca/aP ratio, however, might hamper bone formation because of a Ca deficiency. A Ca/aP ratio of 2.2 was found to be optimal for P efficiency (Van der Klis and Gerritsen, 1994), whereas the Ca/total P ratio should range between 1.5 and 2.2 (Dieckmann, 2004).

It was hypothesized that, because of selection for body gain and feed efficiency, the development of the skeleton in fast growing broilers cannot keep pace with the gain of the soft tissues, muscles and viscera. As a consequence, the skeleton development in modern broilers could be relatively retarded, as shown by a low relative skeleton weight (% of EBW), a low Ca and P content in the skeleton, reduced bone strength, and increased locomotion problems. Moreover, P requirement would be underestimated because it might be assessed upon an immature skeleton.

A better understanding of animal responses to dietary P in interaction with interfering factors such as dietary aP level, Ca/aP ratio, and growth rate (GR) thus represents a prerequisite in order to establish new recommendations. Therefore, an experiment was performed that aimed to determine the effects of two aP levels, three Ca/aP ratios and two growth rates on Ca, P and N deposition in soft tissue and skeleton, tibia development and locomotion properties, digestibility of N, Ca and P, and phosphate excretion in male and female Ross 308 broilers.

2 Material and methods

2.1 Housing, birds and management

A total of 1440 one-day-old Ross-308 broilers were allotted to 48 pens (30 birds per pen). This experiment comprised 12 dietary treatments, as shown in Table 1, indicating that each treatment was replicated 4 times. In two replicate floor pens male chicks and in the other two replicate floor pens female chicks were housed.

Table 1. Overview of dietary treatments applied in the experiment.

Treatment	aP level (% of CVB)[1]	Ca/aP ratio	Growth Rate (GR)
1	-20	1.5	-20% of Ross
2	-20	2.2	-20% of Ross
3	-20	2.9	-20% of Ross
4	+20	1.5	-20% of Ross
5	+20	2.2	-20% of Ross
6	+20	2.9	-20% of Ross
7	-20	1.5	Standard Ross
8	-20	2.2	Standard Ross
9	-20	2.9	Standard Ross
10	+20	1.5	Standard Ross
11	+20	2.2	Standard Ross
12	+20	2.9	Standard Ross

[1]) aP +20% is 3.7 g/kg from d10-30 and 3.4 g/kg from d30-49; aP -20% is 2.5 g/kg from d10-30 and 2.3 g/kg from d30-49 (CVB, 2012).

The birds were fed a low (20% below CVB requirement) or a high (20% above CVB requirement) available phosphorus (aP) level (CVB, 2012). Three dietary Ca/aP ratios were applied (1.5, 2.2 and 2.9 g/g). The broilers were raised according to two growth curves; a slow and a standard growth curve. The slow growing birds were aimed to have a 20% reduced growth rate (GR) compared to the standard growing birds (Ross, 2012). The target final slaughter weight for both growth rates was 2.3 kg.

At d1, 30 one-day old broiler chicks of the same sex were placed in each floor pen (1.50 x 1.00 m). All pens were located in one room in a climate-controlled poultry house. Each floor pen contained a feeding trough (1.50 m wide) and two nipple drinkers, while wood shavings were used as litter. In the starter period (d1 to d10) a standard commercial starter diet was fed to all broilers. At d10, one bird per pen was killed and dissected for determination of N and P contents. From d10 onwards, the respective experimental diets were fed. The standard growing broilers were fed *ad libitum*, whereas the slow growing broilers were fed restrictedly. They received diets, in which the crude protein and amino acid contents were reduced by 12 – 13% compared to the diets fed to the standard growing birds. All broilers had *ad libitum* access to water.
The temperature and relative humidity of the poultry house was continuously registered. The housing temperature was adjusted to the age of the broilers. At d1, 7, 14, 21 and 28, the temperature was set at 34, 28, 25, 22 and 20 °C, respectively. The lights were continuous on during the first two days, there after the birds were subjected to a regime of 16 hours of light and 8 hours of darkness (16 L:8 D). During the 3-d faeces collection period, a continuous light regime was applied. The light intensity was 20 lux.
At d24, 24 standard growing broilers were moved from each floor pen to two adjacent cages (0.65 x 0.75 m), which enabled faeces collection. Because of the smaller size of the cages, the birds were divided over two cages, although these two cages still were considered as one experimental unit. The birds were allowed an one week adaptation period before a 3-d main period (d32, 33, 34) of faeces

collection started. The slow growing birds were moved to similar cages at d38, where after the faeces collection period was performed from d40 to d42. The adaptation period was shorter compared to the standard growing birds in order to prevent large differences in dissection weight between both groups. After each faeces collection period, all birds in the cages were dissected for further observations.
The 5 remaining birds in each floor pen were killed and dissected at a slaughter weight of about 2300 g.
The weight development of the standard and slow growing birds over time is shown in Figure 1.

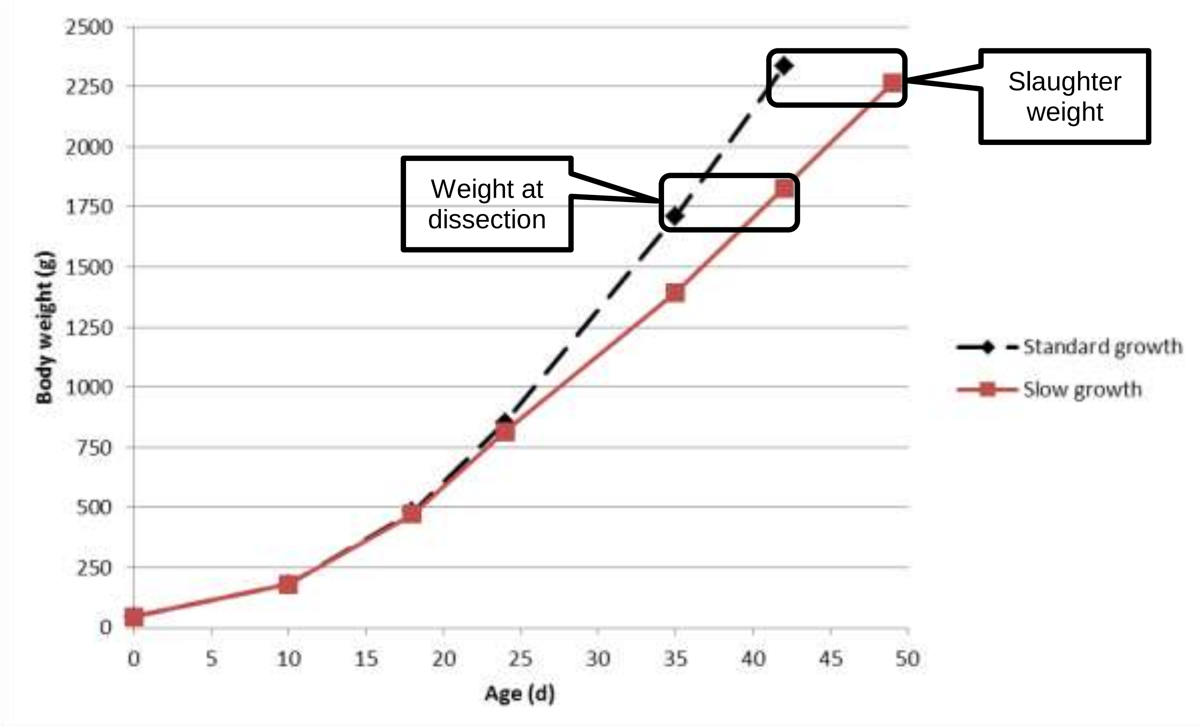

Figure 1. Weight development of standard and slow growing birds over time.

2.2 Experimental design

The experiment had a 2 x 3 x 2 x 2 factorial arrangement. The factors were aP-level (-20%; +20%) (CVB, 2012), Ca/aP-ratio (1.5, 2.2, 2.9), growth rate (standard, low) (Ross, 2012), and sex (male, female), as shown in Table 1. The recommend dietary aP content by CVB is 4.0 g/kg (d1 to d10), 3.1 g/kg (d10 – d30) and 2.8 g/kg (d30 – d40), respectively (CVB, 2012).

The broilers were fed three diets according to a three phase feeding programme: phase one from d1 to d10, phase two from d10 to a BW of about 1750 g (d35 fast growers; d43 slow growers) and phase three from 1750 g to slaughter weight (about 2300 g; d42 fast growers; d49 slow growers). The diet composition of the grower and finisher diets (diet 1, 6, 7 and 12) is shown in Table 2 and 3, respectively. The experimental diets were a mixture of a low or a high protein basal diet, supplemented with limestone (as a pure Ca source), monocalcium phosphate (as a combined Ca and P source) and diamol (as filling component). The contents of limestone, monocalcium phosphate, the calculated contents of Ca and P, and the analysed contents of DM, ash, Ca and P of the 12 grower and 12 finisher diets are shown in Table 4. On average, analysed Ca and P contents were 0.8 g/kg and 0.5 g/kg higher than the calculated values, respectively.

Table 2. Dietary ingredients (g/kg) and calculated contents (g/kg or MJ/kg) of grower diets 1, 6, 7 and 12.

		Diet 1	Diet 6	Diet 7	Diet 12
Maize		650.0	650.0	650.0	650.0
Soybean meal		120.8	120.7	233.7	233.7
Sunflower seed expeller		53.0	53.0	0.0	0.0
Peas		50.0	50.0	0.0	0.0
Fish meal		30.0	30.0	30.0	30.0
Diamol		22.9	0.0	22.9	0.0
Maize gluten meal		20.0	20.0	12.1	12.1
Palm oil		17.9	17.9	16.6	16.6
Soya oil		15.0	15.0	15.0	15.0
Monocalcium phosphate.1H_2O		5.1	11.9	4.7	11.5
Premix[1]		5.0	5.0	5.0	5.0
Salt		3.4	3.4	3.4	3.4
L-Lysine HCl		2.4	2.4	1.6	1.6
Limestone		2.1	18.2	2.2	18.2
DL-Methionine		1.1	1.1	1.5	1.5
Chromium premix		1.0	1.0	1.0	1.0
L-Threonine		0.4	0.4	0.3	0.3
DM	g	881	880	880	880
Ash	g	59	58	61	60
Crude protein	g	176	176	199	199
Crude fat	g	71	71	70	70
Crude fibre	g	36	36	23	23
Starch	g	418	418	398	398
Sugars	g	25	25	33	33
NSP	g	132	132	120	120
Ca	g	3.6	10.5	3.6	10.5
P	g	5.0	6.5	4.9	6.4
aP	g	2.4	3.6	2.4	3.6
K	g	6.7	6.7	7.9	7.9
Na	g	1.7	1.7	1.7	1.7
Cl	g	3.5	3.5	3.3	3.3
ME	MJ	12.20	12.20	12.20	12.20
dig. LYS	g	8.6	8.6	9.9	9.9
dig. MET	g	4.0	4.0	4.7	4.7
dig. M+C	g	6.3	6.3	7.2	7.2
dig. THR	g	5.6	5.6	6.4	6.4
dig. TRP	g	1.5	1.5	1.9	1.9
dig. ILE	g	6.0	6.0	7.2	7.2

[1]) Composition of premix per kg of diet: 12000 IU vitamin A, 2400 IU vitamin D3, 30 IU vitamin E, 1.5 mg vitamin K3, 2 mg vitamin B1, 7.5 mg vitamin B2, 10 mg d-pantothenic acid, 35 mg niacin amide, 200 µg biotin, 20 µg vitamin B12, 1 mg folic acid, 3.5 mg vitamin B6, 460.83 mg choline chloride, 80 mg Fe (as FeSO4.H2O), 12 mg Cu (as CuSO4.5H2O), 60 mg Zn (as ZnSO4.H2O), 85 mg Mn (as MnO), 0.4 mg Co (as CoSO4.7H2O), 0.8 mg I (as KI), 0.1 mg Se (as Na2SeO3) and 50 mg anti-oxidant.

Table 3. Dietary ingredients (g/kg) and calculated contents (g/kg or MJ/kg) of finisher diet 1, 6, 7 and 12.

		Diet 1	Diet 6	Diet 7	Diet 12
Maize		645.0	645.0	645.0	645.0
Soybean meal		175.0	175.0	258.7	258.7
Sunflower seed expeller		43.1	43.1	0.0	0.0
Peas		40.0	40.0	0.0	0.0
Diamol		20.6	0.0	20.6	0.0
soya oil		20.0	20.0	18.0	18.0
Palm oil		19.2	19.2	18.1	18.1
Maize gluten meal		15.0	15.0	17.4	17.4
Monocalcium phosphate.1H_2O		5.6	11.8	5.3	11.5
Premix		5.0	5.0	5.0	5.0
Salt		4.1	4.1	4.1	4.1
L-Lysine HCl		2.5	2.5	2.3	2.3
Limestone		2.3	16.8	2.4	16.9
DL-Methionine		1.3	1.3	1.6	1.6
Chromium premix		1.0	1.0	1.0	1.0
L-Threonine		0.4	0.3	0.4	0.4
Calculated contents					
DM	g	880	880	880	880
Ash	g	57	55	58	57
Crude protein	g	172	172	193	193
Crude fat	g	75	75	72	72
Crude fibre	g	34	34	24	24
Starch	g	411	411	396	396
Sugars	g	30	30	36	36
NSP	g	135	135	124	124
Ca	g	3.3	9.4	3.3	9.4
P	g	4.7	6.1	4.6	6.0
aP	g	2.2	3.3	2.2	3.3
K	g	7.2	7.2	8.1	8.1
Na	g	1.7	1.7	1.7	1.7
Cl	g	3.5	3.5	3.4	3.4
ME	MJ	12.20	12.20	12.20	12.20
dig. LYS	g	8.4	8.4	9.6	9.6
dig. MET	g	3.8	3.8	4.4	4.4
dig. M+C	g	6.1	6.1	7.0	7.0
dig. THR	g	5.5	5.5	6.2	6.2
dig. TRP	g	1.5	1.5	1.8	1.8
dig. ILE	g	6.0	6.0	7.0	7.0

[1]) Composition of premix per kg of diet: 12000 IU vitamin A, 2400 IU vitamin D3, 30 IU vitamin E, 1.5 mg vitamin K3, 2 mg vitamin B1, 7.5 mg vitamin B2, 10 mg d-pantothenic acid, 35 mg niacin amide, 200 µg biotin, 20 µg vitamin B12, 1 mg folic acid, 3.5 mg vitamin B6, 460.83 mg choline chloride, 80 mg Fe (as FeSO4.H2O), 12 mg Cu (as CuSO4.5H2O), 60 mg Zn (as ZnSO4.H2O), 85 mg Mn (as MnO), 0.4 mg Co (as CoSO4.7H2O), 0.8 mg I (as KI), 0.1 mg Se (as Na2SeO3) and 50 mg anti-oxidant.

The main ingredients of the basal diet were maize (650 g/kg) and soybean meal. Chromium (170 mg/kg) was included in the diets as indigestible marker.

Table 4. Contents of diamol, limestone and monocalcium phosphate, calculated contents of Ca, P and Ca/aP, and analysed contents of DM, ash, Ca and P in the grower and finisher diets.

	Dietary ingredients			Calculated contents			Analysed contents			
Grower diets	Diamol (g/kg)	Limestone (g/kg)	Monocalcium phosphate (g/kg)	Ca (g/kg)	P (g/kg)	Ca/aP	DM (g/kg)	Ash (g/kg)	Ca (g/kg)	P (g/kg)
Diet 1	22.90	2.07	5.08	3.6	5.0	1.5	900.0	61.1	4.42	5.93
Diet 2	18.18	6.79	5.08	5.3	5.0	2.2	899.9	60.2	6.27	5.61
Diet 3	13.46	11.51	5.08	7.0	5.0	2.9	900.5	60.2	8.23	5.62
Diet 4	14.18	3.97	11.90	5.4	6.5	1.5	900.5	61.6	6.04	7.13
Diet 5	7.10	11.05	11.90	7.9	6.5	2.2	899.5	59.7	9.14	7.23
Diet 6	0.00	18.15	11.90	10.5	6.5	2.9	900.2	61.6	11.75	7.00
Diet 7	22.90	2.17	4.66	3.6	5.0	1.5	898.7	64.0	3.87	5.36
Diet 8	18.18	6.89	4.66	5.3	5.0	2.2	899.3	62.1	5.98	5.37
Diet 9	13.46	11.61	4.66	7.0	5.0	2.9	898.6	62.9	7.89	5.38
Diet 10	14.19	4.06	11.48	5.4	6.5	1.5	898.3	62.8	5.75	6.58
Diet 11	7.11	11.14	11.48	7.9	6.5	2.2	899.2	62.3	8.44	6.53
Diet 12	0.00	18.25	11.48	10.5	6.5	2.9	899.7	63.4	11.05	6.74
Finisher diets										
Diet 1	20.65	2.26	5.63	3.3	4.7	1.5	897.6	58.2	3.56	5.10
Diet 2	16.38	6.53	5.63	4.8	4.7	2.2	897.8	59.7	5.15	5.04
Diet 3	12.11	10.80	5.63	6.3	4.7	2.9	897.9	59.3	6.70	5.12
Diet 4	12.78	4.00	11.76	4.9	6.1	1.5	898.3	58.1	5.48	6.64
Diet 5	6.38	10.40	11.76	7.2	6.1	2.2	898.5	58.6	7.27	6.35
Diet 6	0.00	16.78	11.76	9.4	6.1	2.9	898.5	61.4	9.50	6.36
Diet 7	20.65	2.37	5.34	3.3	4.7	1.5	899.0	63.4	3.86	5.09
Diet 8	16.38	6.64	5.34	4.8	4.7	2.2	899.0	62.1	5.59	5.19
Diet 9	12.11	10.91	5.34	6.3	4.7	2.9	899.6	63.1	7.68	5.30
Diet 10	12.78	4.10	11.48	4.9	6.1	1.5	899.3	61.2	6.24	6.94
Diet 11	6.37	10.51	11.48	7.2	6.1	2.2	899.3	62.6	8.41	6.99
Diet 12	0.00	16.88	11.48	9.4	6.1	2.9	900.1	61.9	11.85	6.90

2.3 Dissection of birds

At the day of the arrival of the one-day-old broilers, randomly 5 hens and 5 roosters were killed, dissected, autoclaved and ground for chemical analysis. Because it was not possible to grind the large wing feathers homogenously, before grinding these feathers were removed and weighed. The contents of N, Ca and P of these feathers were assumed to be 132.8, 5.0 and 2.7 g/kg, respectively (CVB, 2010). The amount of N, Ca and P contributed by these feathers were added to the nutrient amounts analysed in the carcass. Likewise, at d10, one bird from every pen was dissected for determination of carcass contents. After the period of faeces collection (d35 for the standard growers and d43 for the slow growers) all cage housed birds (24 per pen) were dissected, while the intestinal contents were collected and weighed. The ileal content from the birds of the four cages with the same treatment and gender were pooled to one sample. After dissection and autoclaving, 5 birds of each pen were manually divided in soft tissue and carcass. The soft tissues and carcasses of these 5 birds were pooled, respectively. The tibia of 5 other dissected birds per pen were removed from the carcass and weighed. At the final slaughter age (d42 for the standard growers and d49 for the slow growers) the intestinal contents of the 5 remaining birds in the floor pens were removed, weighed and pooled to one sample. Pooled samples of 8 birds at final slaughter weight (2 birds of each pen with the same dietary treatment, males and females combined) were taken for determination of carcass composition. The dissected birds were analysed for DM, N, crude fat, ash, Ca en P.

2.4 Observations

Body weight and feed intake of the standard growing broilers was determined at d1, 10, 18, 24, 35 and 42. The body weight was determined by measuring all animals per floor pen or cage. In the cages, feed intake was determined over the period from d24 to d30. For slow growing broilers the feed intake and body weight was measured at d1, 10, 18, 24, 35, 38, 43 and 49. Feed conversion ratio was calculated on the basis of feed intake and BW.

At d21 and 28, the gait score was determined using the method of Kestin et al. (1992). The score ranged from 0 for a broiler walking normally and in balance, to 5 for a bird that could not walk anymore. Furthermore, at d 28, the food pad lesions were assessed according the method described by Berg (1998).

Excreta collection and apparent digestibility calculations

Representative samples of excreta were collected semi quantitatively during 3 consecutive days of 24 h (d32, 33 and 34, or d40, 41 and 42 for the standard and slow growing broilers, respectively) to determine faecal apparent digestibility of P and Ca. The calculation of faecal digestibility of these minerals was based on the amount of minerals consumed by feed and on the amount of minerals excreted by the faeces. In addition, the ileal apparent digestibility of DM, N, Ca and P was determined based on chyme collection at slaughter. Excreta and ileal chyme samples were frozen and stored at -18°C until chemical analysis. The apparent ileal digestibility (AID) was calculated with chromium as marker by using the following formula:

$$AID\ (\%) = \{1 - [Cr_{fd}/Cr_{fc} * N_{fc}/N_{fd})]\} * 100$$

where Cr_{fd} is the chromium present in the feed and Cr_{fc} in the ileal chymus or excreta. The N_{fc} and N_{fd} are respectively the nutrients in ileal chymus or excreta and feed.

2.5 Analytical procedures

After defrosting, excreta samples were homogenized and subsequently representative samples were taken. All samples were analysed in duplicate. The faeces and feed were freeze-dried according to ISO 6469 (1998a), ground in a 1-mm sieve and kept for analysis. Kjeldahl nitrogen content in feed and ileal chyme was measured according to ISO 5983 (1997) in fresh samples. The diets were analysed on DM, ash, N, Ca and P. The chyme was freeze-dried and analysed for Cr, DM, N, Ca and P. The crude protein content was calculated as nitrogen * 6.25. For determining crude ash content, feed was incinerated at 550^0C in a muffle furnace according to ISO 5984 (2002). Minerals were determined according to ISO 11885(1998b) and chromium oxide according to the method described by Williams et al (1962).

The strength of the tibia was measured with an Instron Materials tester (model 5564) with automated materials test software (Figure 2). The breaking point was defined as the point at which the resistance of the bone was reduced by 30% or as the bone was bent by 10 mm. The power used to break the bone was registered 9 times/s by the software of the Instron Materials tester. The total breaking strength was expressed in Nmm. The tibia diameter was measured by the material tester Heidenhain. After breaking the tibia, these bones were autoclaved for six hours and grinded, after which a pooled sample of the 5 tibiae per pen were analysed for ash, Ca, P and DM.

Figure 2. Instron materials tester, model 5564, used for measuring the breaking strength of the tibia.

2.6 Data analysis

A REML ((Restricted Maximum Likelihood) variance component analysis procedure (Genstat 8 Committee, 2002) was used to test the effects of the nutritional factors on the determined traits, using the model:

Y_{ijkl} = μ + Ca/aP $ratio_i$ * aP-$level_j$ * growth $rate_k$ * sex_l + e_{ijkl}

Where Y_{ijkl} = dependent variable; μ = overall mean; Ca/aP $ratio_i$ = fixed effect of season (i = 3, 1.5, 2.2 and 2.9)); aP-$level_j$ = fixed effect of aP-level (j=2, 20% below and 20% above CVB requirement); growth $rate_k$= fixed effect of growth rate (k=2, standard growth rate and 20% below the standard); sex_l = fixed effect of sex (l=2, male and female); e_{ijkl} = error term. Pen was added as a random term.

A *P*-value below 0.05 was considered to be significant.

3 Results

This section provides the results regarding body composition of 1750 g broilers, subdivided in contents in soft tissue, skeleton and whole empty body, the measured characteristics of the tibia, some locomotion traits, ileal and faecal nutrient digestibility, and finally the performance traits. The results of the main effects are shown in Table 5 to Table 9. Significant interaction effects are shown in Table 10 to Table 18.

3.1 Contents in soft tissue (broiler weight about 1750 g)

The ash and Ca contents of the soft tissue were not affected by the treatments (Table 5). The P content in the soft tissue was only affected by the GR (P = 0.019). Birds with a standard GR had a higher P content in the soft tissue than birds with a low GR (2.36 vs. 2.18 g/kg).
If birds were fed a high dietary aP level, Ca/aP ratio did not affect the N content of soft tissue, but in case of feeding a low dietary aP level the N content of soft tissue was increased at the standard Ca/aP ratio (32.7 g/kg) compared to the high (28.4 g/kg) and the low (29.9) Ca/aP ratio (Table 11). The N content in the soft tissue was affected by a GR * aP * sex interaction. In almost all treatments, males had a higher or similar N content in soft tissue than females. Females, however, with a standard growth rate and fed on a high aP level had a higher N content in soft tissue compared to the males with the same treatment (32.1 vs. 30.0 g/kg; Table 12).
Birds with the standard GR contained more fat in the soft tissue compared to birds with the low GR (119.5 vs. 114.8 g/kg). Females had a higher fat content in soft tissue than males (124.4 vs. 110.0 g/kg).

3.2 Skeleton characteristics

Skeleton portion was highest in birds fed with the standard Ca/aP ratio (8.2% of EBW) and lowest in birds fed with the low Ca/aP ratio (7.0 % of EBW), whereas portion of the high Ca/aP ratio was in between (7.6% of EBW) (Table 6). Skeleton portion was 15% higher in birds with the low GR compared to the standard GR (8.2 vs. 7.1% of EBW). Feeding a high aP level resulted in a 10% increase in skeleton portion (8.0 vs. 7.3 % of EBW). Skeleton portion of males was 7% higher compared to females (7.9 vs. 7.4% of EBW).
The ash content in the skeleton increased with increasing Ca/aP ratio and aP level, but was not affected by GR and sex.
The Ca content of the skeleton tended (P = 0.062) to be lower in the birds fed the low Ca/aP ratio (59.6 g/kg) compared to the birds fed the standard and high Ca/aP ratio's (64.6 g/kg). The Ca content of the skeleton also tended (P = 0.062) to be higher in birds fed the high compared to the low aP level (64.7 vs. 61.3 g/kg). Skeletons of females contained more Ca compared to skeletons of males (64.8 vs. 61.0 g/kg).
The P content of the skeleton was not affected by Ca/aP ratio, GR and Sex, whereas feeding a high dietary aP level resulted in an increased P content of the skeleton compared to feeding a low aP level (33.8 vs. 31.3 g/kg).
Among treatments, 75.3% of body Ca is deposited in the skeleton. Feeding a standard Ca/aP ratio resulted in a higher Ca portion in the skeleton (78.6%) compared to feeding a high or low Ca/aP ratio (on average 73.7%). In the slow growing birds, 77.5% of body Ca was deposited in the skeleton versus 73.1% in the standard growing birds. Feeding the high aP level resulted in a higher Ca portion in the skeleton compared to feeding the low aP level (77.4 vs. 73.2%).
Among treatments, 53.6% of body P is deposited in the skeleton. Feeding a standard Ca/aP ratio resulted in a higher P portion in the skeleton (56.2%) compared to feeding a high (54.2%) or a low (50.4%) Ca/aP ratio. In the slow growing birds, 56.4% of body P was deposited in the skeleton versus 50.9% in the standard growing birds. Feeding the high aP level resulted in a higher P portion in the skeleton compared to feeding the low aP level (56.3 vs. 51.0%).

Table 5. Effects of dietary treatments on contents of ash, Ca, P, N and fat in soft tissue, and content of P and N in EBW (g/kg) at 1750 g body weight.

	Ash content soft tissue (g/kg)	Ca content soft tissue (g/kg)	P content soft tissue (g/kg)	N content soft tissue (g/kg)	Fat content soft tissue (g/kg)	P content EBW (g/kg)	N content EBW (g/kg)
Ca/aP ratio							
High	15.6	1.78	2.28	29.5	115.7	4.67	28.7
Standard	16.0	1.49	2.25	31.7	120.3	4.79	30.7
Low	15.4	1.60	2.29	30.3	115.6	4.37	29.6
SEM	0.52	0.11	0.06	0.52	2.00	0.07	0.45
Growth Rate							
Standard	15.7	1.66	2.36	31.0	119.5	4.55	30.3
Low	15.7	1.58	2.18	30.0	114.8	4.67	29.0
SEM	0.42	0.09	0.05	0.42	1.60	0.05	0.37
aP-level							
High	15.8	1.58	2.24	30.7	117.9	4.79	29.6
Low	15.5	1.66	2.31	30.3	116.4	4.44	29.7
SEM	0.42	0.09	0.05	0.42	1.60	0.05	0.37
Sex							
Male	15.4	1.66	2.30	30.6	110.0	4.71	29.8
Female	15.9	1.58	2.24	30.4	124.4	4.51	29.5
SEM	0.42	0.09	0.05	0.42	1.60	0.05	0.37
P-values							
Ca/aP ratio	0.663	0.207	0.893	**0.019**	0.174	**<.001**	**0.016**
Growth Rate	0.999	0.586	**0.019**	0.093	**0.049**	0.107	**0.016**
aP-level	0.628	0.562	0.361	0.567	0.513	**<.001**	0.955
Sex	0.428	0.586	0.371	0.682	**<.001**	**0.018**	0.466
Ca/aP *GR	0.325	0.280	0.135	0.076	0.945	**0.010**	0.084
Ca/aP *aP	0.627	0.165	0.154	**0.025**	0.584	0.583	**0.016**
Ca/aP *Sex	0.594	0.656	0.482	0.984	0.582	0.099	0.914
GR *aP	0.999	0.795	0.670	0.617	0.583	0.739	0.675
GR *Sex	0.393	0.317	0.575	0.461	0.714	0.969	0.593
aP *Sex	0.234	0.345	0.261	0.762	0.829	0.443	0.485
Ca/aP *GR*aP	0.281	0.609	0.685	0.283	0.523	0.420	0.196
Ca/aP *GR*Sex	0.151	0.922	0.698	0.573	0.151	0.280	0.575
Ca/aP *aP *Sex	0.961	0.295	0.338	0.261	0.185	0.097	0.307
GR * aP * Sex	0.168	0.739	0.202	**0.007**	0.084	**0.045**	**0.005**
Ca/aP *GR*aP *Sex	0.151	0.595	0.866	**0.040**	0.451	0.969	0.072

Table 6. Effects of treatments on skeleton portion (% of EBW), and its contents of ash, Ca and P, portion of P and Ca deposited in it, and Ca and P contents in bone ash (g/kg).

	Skeleton portion (% of EBW)	Ash content skeleton (g/kg	Ca content skeleton (g/kg)	P content skeleton (g/kg)	Ca portion in skeleton (%)[1]	P portion in skeleton (%)[1]	Ca content in skeleton ash (g/kg)	P content in skeleton ash (g/kg)
Ca/aP ratio								
High	7.6	190.5	64.8	33.2	74.4	54.2	341.0	174.4
Standard	8.2	186.2	64.3	32.8	78.6	56.2	347.1	176.1
Low	7.0	176.2	59.6	31.5	73.0	50.4	339.4	179.1
SEM	0.17	3.9	1.63	0.73	1.55	0.95	9.2	1.3
Growth Rate								
Standard	7.1	185.3	62.3	32.9	73.1	50.9	339.0	177.6
Low	8.2	183.3	63.4	32.2	77.5	56.4	346.0	175.5
SEM	0.14	3.2	1.33	0.60	1.26	0.78	7.5	1.0
aP-level								
High	8.0	192.1	64.7	33.8	77.4	56.3	337.8	175.7
Low	7.3	176.5	61.3	31.3	73.2	51.0	347.2	177.4
SEM	0.14	3.2	1.33	0.60	1.26	0.78	7.5	1.0
Sex								
Male	7.9	183.7	61.0	32.5	74.6	53.9	333.9	176.9
Female	7.4	184.9	64.8	32.6	76.0	53.3	351.1	176.2
SEM	0.14	3.2	1.33	0.60	1.26	0.78	7.5	1.0
P-values								
Ca/aP ratio	**<.001**	**0.046**	0.062	0.256	**0.043**	**<.001**	0.825	**0.042**
Growth Rate	**<.001**	0.669	0.560	0.416	**0.022**	**<.001**	0.511	0.170
aP-level	**<.001**	**0.002**	0.062	**0.007**	**0.029**	**<.001**	0.381	0.253
Sex	**0.027**	0.792	**0.051**	0.961	0.439	0.584	0.117	0.600
Ca/aP *GR	0.891	0.646	0.650	0.262	0.633	0.963	0.663	**0.026**
Ca/aP *aP	0.412	0.611	0.950	0.473	0.220	0.114	0.646	0.204
Ca/aP *Sex	0.692	0.621	0.775	0.719	0.950	0.995	0.990	0.663
GR *aP	0.739	0.750	0.163	0.915	0.369	0.574	0.267	0.152
GR*Sex	0.153	0.391	0.998	0.455	0.247	0.500	0.566	0.766
aP *Sex	0.969	0.643	0.738	0.641	0.511	0.372	0.576	0.975
Ca/aP *GR*aP	0.946	0.644	0.120	0.542	0.544	0.881	0.060	0.485
Ca/aP *GR*Sex	0.961	0.616	0.853	0.447	0.990	0.977	0.818	0.089
Ca/aP *aP *Sex	0.148	0.394	0.410	0.367	0.286	0.444	0.415	0.625
GR * aP * Sex	0.651	0.863	0.798	0.627	0.836	0.766	0.792	**0.051**
Ca/aP *GR*aP *Sex	0.252	0.449	0.345	0.483	0.455	0.740	0.481	0.612

[1]) Portion of total mineral amount in the body that is deposited in the skeleton.

Table 7. Effects of treatments on tibia weight (in g and % of EBW), strength (in Nmm, Nmm/g tibia and Nmm/mm tibia), gait score, and Ca and P in tibia (g/kg).

	Tibia weight (g)	Tibia weight (% of EBW)	Tibia strength (Nmm)	Tibia strength (Nmm/g Tibia)	Tibia strength (Nmm/mm Tibia)	Gait Score d28[1]	Ash content tibia (g/kg)	Ca content tibia (g/kg)	P content tibia (g/kg)
Ca/aP ratio									
High	17.32	1.05	601	35.4	86.2	1.65	466	361	168
Standard	18.57	1.14	539	29.6	78.6	1.67	472	343	162
Low	17.29	1.03	492	29.1	74.4	1.48	444	341	162
SEM		0.022	19.0	0.91	2.35	0.11	2.0	5.1	2.4
Growth Rate									
Standard	16.06	1.01	592	37.1	85.1	1.79	449	341	161
Low	19.39	1.13	496	25.7	74.4	1.40	472	356	167
SEM		0.018	15.5	0.75	1.92	0.09	1.6	4.2	2.0
aP-level									
High	17.94	1.08	553	31.4	80.9	1.65	470	348	164
Low	17.51	1.06	535	31.4	78.6	1.54	451	349	164
SEM		0.018	15.5	0.75	1.92	0.09	1.6	4.2	2.0
Sex									
Male	19.05	1.13	586	31.6	83.2	1.75	455	347	163
Female	16.40	1.01	502	31.2	76.3	1.44	466	350	165
SEM		0.018	15.5	0.75	1.91	0.09	1.6	4.2	2.0
P-values									
Ca/aP ratio	0.084	**0.003**	**0.002**	**<.001**	**0.006**	0.397	**<.001**	**0.019**	0.182
Growth Rate	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**0.004**	**<.001**	**0.018**	**0.050**
aP-level	0.416	0.637	0.444	0.987	0.421	0.368	**<.001**	0.950	0.929
Sex	**<.001**	**<.001**	**<.001**	0.666	**0.017**	**0.019**	**<.001**	0.629	0.658
Ca/aP *GR	0.655	**0.034**	0.130	**0.030**	0.112	0.319	0.231	0.421	0.377
Ca/aP *aP	0.498	0.343	0.136	0.239	0.112	0.781	**0.003**	0.285	0.466
Ca/aP *Sex	0.464	**0.025**	0.060	**0.004**	**0.024**	0.781	0.211	0.196	0.168
GR *aP	0.972	0.135	0.247	0.150	0.394	0.263	0.141	0.227	0.201
GR*Sex	**0.017**	0.318	0.566	0.601	0.122	0.368	0.845	0.222	0.953
aP *Sex	0.403	0.196	0.210	0.488	0.348	0.650	0.655	0.232	0.267
Ca/aP *GR*aP	0.930	0.566	0.082	0.073	0.254	0.621	**0.029**	0.509	0.376
Ca/aP *GR*Sex	0.281	0.192	0.755	0.064	0.671	0.844	0.903	0.134	**0.032**
Ca/aP *aP *Sex	0.527	**0.044**	0.456	0.786	0.406	0.844	0.192	0.286	0.324
GR * aP * Sex	0.767	0.623	0.440	0.517	0.319	0.498	0.854	0.562	0.790
Ca/aP *GR*aP *Sex	0.412	0.426	0.095	0.469	0.088	0.252	**0.019**	0.526	0.621

[1]) Gait score ranges from 0 (broiler walks normally and is in balance) to 5 (broiler cannot walk anymore) (Kestin et al., 1992)

Table 8. Effects of treatments on ileal and faecal nutrient digestibility (%) and phosphate excretion (g/bird/d).

	Ileal DM dig. (%)	Ileal N dig. (%)	Ileal Ca dig. (%)	Ileal P dig. (%)	Faecal Ca dig. (%)	Faecal P dig. (%)	Phosphate excretion (g/bird/d)
Ca/aP ratio							
High	73.9	83.4	31.3	39.6	30.1	36.6	1.02
Standard	72.7	82.7	34.5	42.6	40.3	36.1	0.99
Low	72.3	82.2	51.0	58.1	58.7	36.1	0.98
SEM	0.68	0.63	1.18	1.10	0.78	1.16	0.013
Growth Rate							
Standard	72.7	81.7	43.7	48.3	45.1	37.7	1.03
Low	73.2	83.8	34.2	45.2	40.9	34.8	0.96
SEM	0.55	0.51	0.96	0.89	0.64	0.95	0.011
aP-level							
High	74.1	83.4	29.3	44.9	33.8	31.1	1.21
Low	71.8	82.1	48.6	48.7	52.2	41.4	0.79
SEM	0.55	0.51	0.96	0.89	0.64	0.95	0.011
Sex							
Male	72.5	82.4	38.8	47.3	45.1	37.8	1.01
Female	73.4	83.2	39.1	46.2	40.9	34.7	0.98
SEM	0.55	0.51	0.96	0.89	0.64	0.95	0.011
P-values							
Ca/aP ratio	0.263	0.399	**<.001**	**<.001**	**<.001**	0.942	0.149
Growth Rate	0.579	**0.007**	**<.001**	**0.020**	**<.001**	0.059	**<.001**
aP-level	**0.007**	0.092	**<.001**	**0.006**	**<.001**	**<.001**	**<.001**
Sex	0.249	0.291	0.867	0.388	**<.001**	**0.018**	0.296
Ca/aP *GR	0.993	0.877	0.079	0.431	**0.013**	0.355	0.063
Ca/aP *aP	0.117	0.366	**<.001**	**0.003**	0.737	**0.045**	**0.028**
Ca/aP *Sex	0.896	0.796	0.400	0.994	0.204	0.990	0.093
GR *aP	0.365	0.395	0.759	0.178	0.761	0.702	0.127
GR*Sex	0.171	0.176	0.494	0.614	**0.036**	0.150	**<.001**
aP *Sex	0.945	0.756	0.965	0.692	**0.028**	0.935	0.974
Ca/aP *GR*aP	0.076	0.285	**0.008**	0.125	**0.007**	0.433	**0.041**
Ca/aP *GR*Sex	0.790	0.726	0.728	0.471	0.437	0.798	0.860
Ca/aP *aP *Sex	0.326	0.555	0.235	0.806	**0.007**	0.098	0.155
GR*aP * Sex	0.984	0.980	0.468	0.470	0.579	0.828	0.844
Ca/aP *GR*aP *Sex	0.453	0.683	0.620	0.820	0.656	0.916	0.855

Table 9. Effects of treatments on performance characteristics of the birds

	Bird weight d35 (g)	Bird weight d42 (g)	Bird weight d49 (g)[1]	Feed intake d10 – d42 (g/d)	Daily gain d10 – d42 (g/d)	FCR d10 – d42 (g/g)
Ca/aP ratio						
High	1560	2096	2260	99.9	59.9	1.682
Standard	1549	2117	2310	100.2	60.5	1.672
Low	1508	2029	2221	97.8	57.8	1.710
SEM	13.7	27.7	35.5	0.76	0.87	0.020
Growth Rate						
Standard	1684	2333	- - -	107.9	67.3	1.610
Low	1394	1828	2264	90.6	51.5	1.767
SEM	11.2	22.6	- - -	0.62	0.71	0.017
aP-level						
High	1556	2129	2315	99.8	60.9	1.655
Low	1522	2032	2212	98.8	57.8	1.721
SEM	11.2	22.6	29.0	0.62	0.71	0.017
Sex						
Male	1578	2165	2365	102.8	62.1	1.672
Female	1500	1996	2162	95.8	56.6	1.704
SEM	11.2	22.6	29.0	0.62	0.71	0.017
P-values						
Ca/aP ratio	**0.031**	0.083	0.249	0.074	0.091	0.411
Growth Rate	**<.001**	**<.001**	- - -	**<.001**	**<.001**	**<.001**
aP-level	**0.040**	**0.005**	**0.027**	0.293	**0.006**	**0.010**
Sex	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	0.186
Ca/aP *GR	0.211	0.910	- - -	0.590	0.915	0.796
Ca/aP *aP	0.740	0.911	0.671	0.347	0.905	0.490
Ca/aP *Sex	0.911	0.852	0.514	0.926	0.865	0.700
GR *aP	0.275	0.569	- - -	0.916	0.550	0.907
GR*Sex	**0.012**	0.648	- - -	0.178	0.619	0.287
aP *Sex	0.855	**0.047**	0.116	0.669	**0.048**	**0.038**
Ca/aP *GR*aP	0.739	0.931	**- - -**	0.573	0.929	0.435
Ca/aP *GR*Sex	0.716	0.268	- - -	0.843	0.274	0.076
Ca/aP *aP *Sex	0.787	0.373	0.608	0.593	0.372	0.687
GR * aP * Sex	0.980	0.708	- - -	0.416	0.731	0.584
Ca/aP *GR*aP *Sex	0.476	0.096	- - -	0.281	0.099	0.354

[1] The values in this column only concern the slow growing birds.

The experimental factors did not affect the Ca content in the skeleton ash. The P content in the skeleton ash was inversely related to the Ca/aP ratio and decreased from 179.1 g/kg in birds fed a low Ca/aP ratio to 174.4 g/kg in birds fed a high Ca/aP ratio. The P content in the skeleton ash was affected by a GR * aP * sex interaction (Table 12). The P content in skeleton ash was significantly increased in standard growing females fed diets with a low aP level (181.1 g/kg) and significantly decreased in standard growing females fed diets with a high aP level (173.6 g/kg), whereas all other treatment combinations did not differ from each other (on average 176.3 g/kg).

Table 10. Interaction effects of Ca/aP ratio and Growth Rate (GR) on tibia weight (% of EBW) and tibia strength (Nmm/g tibia).

Treatment	Tibia weight (% of EBW)	Tibia strength (Nmm/g tibia)
High Ca/aP		
GR Standard	0.95^{b}	43.1^{a}
GR Low	1.14^{a}	27.7^{c}
Standard Ca/aP		
GR Standard	1.12^{a}	34.9^{b}
GR Low	1.15^{a}	24.3^{d}
Low Ca/aP		
GR Standard	0.94^{b}	33.2^{b}
GR Low	1.11^{a}	25.1^{cd}
SE	0.031	1.29
P-value	**0.034**	**0.030**

Table 11. Interaction effects of Ca/aP ratio and aP level on N content in soft tissue and EBW (g/kg) and on ileal and faecal P digestibility (%)

Treatment	N content soft tissue (g/kg)	N content EBW (g/kg)	Ileal P digestibility (%)	Faecal P digestibility (%)
High Ca/aP				
aP High	30.6^{b}	29.6^{b}	37.4^{d}	28.9^{d}
aP Low	28.4^{b}	27.4^{c}	41.7^{c}	44.2^{a}
Standard Ca/aP				
aP High	30.7^{b}	29.6^{b}	43.8^{c}	31.6^{cd}
aP Low	32.7^{a}	31.7^{a}	41.4^{c}	40.6^{b}
Low Ca/aP				
aP High	30.8^{b}	29.6^{b}	53.4^{b}	32.9^{c}
aP Low	29.9^{b}	29.5^{b}	62.9^{a}	39.3^{b}
SE	0.73	0.64	1.55	1.64
P-value	**0.025**	**0.016**	**0.003**	**0.045**

3.3 Contents in Empty Body Weight (EBW) (broiler weight about 1750 g)

Average P content in EBW among treatments was 4.6 g/kg. Applying the standard or high Ca/aP ratio resulted in a higher P content in EBW compared to the low Ca/aP ratio (4.73 vs. 4.37 g/kg), as shown in Table 5. The P content in EBW was affected by a GR * aP * sex interaction. Details of relevant treatment combinations are presented in Table 12. In birds with the standard GR, P content in EBW was similar for males and females if the high aP level was supplied (4.7 g/kg), whereas the P content was reduced in females compared to the males if the low aP level was supplied (4.6 vs. 4.2 g/kg). In birds with the low GR, P content was increased in males compared to the females if the high aP level was supplied (5.0 vs. 4.7 g/kg), whereas no sex effect occurred if the low aP level was supplied (4.5 g/kg).

The N content in EBW was affected by a Ca/aP ratio * aP interaction (Table 11). The N content was not affected by Ca/aP ratio if a high aP level was supplied (29.6 g/kg), whereas in case of the low aP level the N content was increased at the standard Ca/aP ratio (31.7 g/kg) or reduced at the high Ca/aP ratio (27.7 g/kg). The N content in EBW was affected by a GR * aP * sex interaction (Table 12). In birds with the standard GR, N content in EBW was increased in females compared to males if the high aP level was supplied (31.1 vs. 29.3 g/kg), whereas the N content was increased in males compared to females if the low aP level was supplied (31.5 vs. 29.4 g/kg). In birds with the low GR, N content was increased in males compared to females if a high aP level was supplied (30.0 vs. 28.1 g/kg), whereas no sex effect occurred if the low aP level was supplied (28.9 g/kg).

3.4 Tibia characteristics and gait score

Weight of tibia among treatments amounted 17.7 g, which is 1.07% of EBW. Relative tibia weight (% of EBW) is affected by an interaction of Ca/aP ratio and GR, as shown in Table 10. In slow growing birds, relative tibia weight was not affected by Ca/aP ratio (1.13%), whereas in standard growing birds the relative tibia weight was reduced in birds that were fed diets with a high (0.95%) or a low (0.94%) Ca/aP ratio compared to birds fed the standard Ca/aP ratio (1.12%). The relative tibia weight was also affected by a Ca/aP * aP * sex interaction (Table 14). In birds fed a diet with a standard or low Ca/aP ratio, relative tibia weight of males was higher compared to females and this difference was not affected by aP level of the diet. In birds fed a diet with a high Ca/aP ratio, relative tibia weight was higher in females compared to males if the diet contained the high aP level (1.06 vs 1.00%), whereas relative tibia weight higher in males compared to females if the diet contained the low aP level (1.14 vs. 0.99 %).

The tibia strength linearly increased with increasing Ca/aP ratio from 492 Nmm at the low Ca/aP ratio to 601 Nmm at the high Ca/aP ratio. The tibia strength was higher in birds with the standard GR (592 Nmm) compared to the birds with the low GR (496 Nmm). Males had a stronger tibia compared to females (586 vs. 502 Nmm).

Tibia strength expressed as Nmm/g tibia was affected by a Ca/aP ratio * GR interaction, as shown in Table 10. In birds with a standard GR tibia strength/g tibia was increased in case of the high Ca/aP ratio (43.1 Nmm/g) compared to the standard and low Ca/aP ratio (34.1 Nmm/g among treatments). In birds with a low GR tibia strength was increased in case of the high Ca/aP ratio compared to the standard Ca/aP ratio (27.7 vs 24.3 Nmm/g), whereas the tibia strength of the low Ca/aP ratio (25.1 Nmm/g) did not significantly differ from the other two treatments. Tibia strength expressed as Nmm/g tibia was also affected by a Ca/aP ratio * sex interaction, as shown in Table 13. In males, tibia strength/g tibia increased by increasing Ca/aP ratio, whereas in females tibia strength/g tibia was similar for the high and standard Ca/aP ratio (32.8 Nmm/g) but was reduced for the low Ca/aP ratio (27.8 Nmm/g).

Tibia strength, expressed as Nmm/mm tibia diameter, was higher in birds with a standard GR compared to birds with the low GR (85.1 vs. 74.4 Nmm/mm tibia). Tibia strength expressed as Nmm/mm tibia was also affected by a Ca/aP ratio * sex interaction, as shown in Table 13. In birds fed the high or low Ca/aP ratio, tibia strength/g tibia was increased in males compared to females (86.4 vs. 74.3 Nmm/g among treatments), whereas tibia strength was not affected by sex in birds fed the standard Ca/aP ratio (78.6 Nmm/g).

Table 12. Interaction effects of growth rate (GR), aP-level and sex on N content of soft tissue, P and N content in EBW (g/kg), and P content in skeleton ash (%).

Treatment	N content soft tissue (g/kg)	P content EBW (g/kg)	N content EBW (g/kg	P content in skeleton ash (%)
GR Standard				
aP High				
Male	30.0[bc]	4.70[b]	29.3[cd]	174.3[bc]
Female	32.1[a]	4.72[b]	31.1[ab]	176.9[bc]
GR Standard				
aP Low				
Male	31.9[a]	4.60[bc]	31.5[a]	181.1[a]
Female	30.1[bc]	4.18[d]	29.4[cd]	177.9[ab]
GR Low				
aP High				
Male	31.5[ab]	5.01[a]	30.0[bc]	177.9[ab]
Female	29.2[c]	4.72[b]	28.1[d]	173.6[c]
GR Low				
aP Low				
Male	29.2[c]	4.53[bc]	28.6[cd]	174.4[bc]
Female	30.1[bc]	4.44[c]	29.2[cd]	176.2[bc]
SEM	0.85	0.108	0.74	2.00
P-value	**0.007**	**0.045**	**0.005**	**0.051**

Table 13. Interaction effects of Ca/aP ratio and sex on tibia strength expressed in Nmm/g tibia and Nmm/mm tibia.

Treatment	Tibia strength (Nmm/g Tibia)	Tibia strength (Nmm/mm Tibia)
High Ca/aP		
Male	37.3[a]	94.3[a]
Female	33.5[b]	78.1[b]
Standard Ca/aP		
Male	27.1[d]	76.9[bc]
Female	32.2[bc]	80.3[b]
Low Ca/aP		
Male	30.5[c]	78.5[b]
Female	27.8[d]	70.4[c]
SEM	1.29	3.33
P-value	**0.004**	**0.024**

The tibia ash content was much higher in slow growing birds compared to standard growers (472 vs. 449 g/kg), although tibia ash was affected by a Ca/aP * aP * GR interaction, as shown in Table 16. In slow growing birds, Ca/aP ratio and aP level did hardly affect tibia ash content (474 – 483 g/kg), except for birds fed the low Ca/aP ratio combined with the low aP level (442 g/kg). In standard growing birds, tibia ash content was increased if birds were fed the high aP level compared to the low aP level, although differences between both aP levels were rather small in birds fed the standard Ca/aP ratio (459 vs. 450 g/kg) compared to birds fed the high Ca/aP ratio (477 vs. 448 g/kg) or the low Ca/aP ratio (444 vs. 415 g/kg).
Tibia of birds fed with the high Ca/oP ratio contained more Ca compared to the tibia of the other two Ca/oP ratios (361 vs. 342 g/kg). Tibia of the slow growing birds contained more Ca compared to standard growing birds (356 vs. 341 g/kg).
The P content of the tibia was affected by a Ca/aP ratio * GR * sex interaction, as shown in Table 15. In female birds fed a diet with a high or standard Ca/aP ratio, GR did not affect the P content of the tibia, whereas in case of the low Ca/aP ratio the P content of the tibia was reduced in standard growing female birds compared to slow growers (170.0 vs. 153.5 g/kg). In male birds fed a diet with a standard or low Ca/aP ratio, GR did not affect the P content of the tibia, whereas in case of the high Ca/aP ratio the P content of the tibia was reduced in standard growing male birds compared to slow growers (179.5 vs. 160.0 g/kg).

Walking characteristics, as expressed by the gait score, were not affected by the treatments at 21 d of age. At 28 d of age, Ca/aP ratio and aP level did not affect the gait score, whereas the gait score was increased in the standard growing birds compared to the slow growers (1.79 vs. 1.40). The gait score was also higher in males than females (1.75 vs. 1.44).

Table 14. Interaction effects of Ca/aP ratio, growth rate (GR) and sex on relative tibia weight (% of EBW) and faecal Ca digestibility (%).

Treatment	Tibia weight (% of EBW)	Faecal Ca Digestibility (%)
Ca/aP High		
aP High		
Male	1.00[d]	20.8[h]
Female	1.06[bc]	20.2[h]
Ca/aP High		
aP Low		
Male	1.14[b]	41.2[e]
Female	0.99[d]	38.3[ef]
Ca/aP Standard		
aP High		
Male	1.25[a]	37.3[f]
Female	1.08[bc]	25.7[g]
Ca/aP Standard		
aP Low		
Male	1.25[a]	49.0[c]
Female	0.96[d]	49.1[c]
Ca/aP Low		
aP High		
Male	1.11[bc]	51.8[c]
Female	0.95[d]	47.3[d]
Ca/aP Low		
aP Low		
Male	1.04[cd]	70.7[a]
Female	1.00[d]	65.1[b]
SEM	0.044	1.55
P-value	**0.044**	**0.007**

3.5 Apparent ileal and faecal nutrient digestibility and phosphate excretion

Feeding a diet with a low aP level reduced ileal DM digestibility compared to feeding a high aP diet (74.1 vs. 71.8%) (Table 8).
Birds with the low GR had a 2.1% higher ileal N digestibility compared to the standard growing birds (83.8 vs. 81.7%).
Ileal Ca digestibility was affected by a Ca/aP ratio * GR * aP interaction, as shown in Table 16. Independent of GR, ileal Ca digestibility of birds fed a high aP diet increased if the Ca/aP ratio decreased: 17.0%, 27.3%, and 43.6% in case of the high, standard and low Ca/aP ratio, respectively. In standard growing birds that were fed the low aP diets, ileal Ca digestibility however, did not differ between the high and standard Ca/aP ratio (on average 51.6%), although ileal Ca digestibility was further increased in the low Ca/aP ratio (58.0%). In slow growing birds that were fed the low aP diets, ileal Ca digestibility was highest if a low Ca/aP ratio was fed (58.8%) and lowest in case of a standard Ca/aP ratio (32.8%), whereas the ileal Ca digestibility of birds fed the high Ca/aP ratio was in between (39.3%).
Ileal P digestibility of standard growing birds was 3.1% higher compared to slow growing birds (48.3 vs. 45.2%, Table 8). The ileal P digestibility was affected by a Ca/aP ratio * aP interaction, as shown in Table 11. A low aP level of the diet increased the P digestibility in birds fed a diet with a high Ca/aP ratio (41.7 vs. 37.4%) or a low Ca/aP ratio (62.9 vs. 53.4%), while aP level did not affect the P digestibility in birds fed a standard Ca/aP ratio (on average 42.6%).
The faecal Ca digestibility was affected by a Ca/aP * GR * aP interaction, as shown in Table 16. Independent of GR, faecal Ca digestibility of birds fed a high aP diet increased if the Ca/aP ratio decreased: 27.2%, 31.5%, and 49.6% in case of the high, standard and low Ca/aP ratio, respectively. In standard growing birds that were fed the low aP diets, faecal Ca digestibility increased as well with decreasing Ca/aP ratio (44.2%, 52.4% and 67.9%, respectively). In slow growing birds that were fed the low aP diets, faecal Ca digestibility was highest if a low Ca/aP ratio was fed (67.9%) and lowest in case of a standard Ca/aP ratio (45.7%), whereas the faecal Ca digestibility of birds fed the high Ca/aP ratio was in between (48.0%).The faecal Ca digestibility was also affected by a Ca/aP ratio * aP * sex interaction, as shown in Table 14. In slow growing birds fed a diet with a standard Ca/aP ratio, faecal Ca digestibility was reduced compared to standard growers, which was independent of sex. In slow growing male birds fed a diet with a high Ca/aP ratio, faecal Ca digestibility was reduced compared to standard growers, but no difference in faecal Ca digestibility was observed in slow growing female birds fed with a high Ca/aP ratio. Independent of aP level, male birds fed a low Ca/oP diet had an increased faecal Ca digestibility compared to female birds (61.3% vs. 56.3%).
Male birds were slightly more efficient in faecal P digestibility (37.8% vs. 34.7%) compared to females. Moreover, faecal P digestibility was effected by a Ca/aP *aP interaction (Table 11). In birds fed diets with a high aP level, faecal P digestibility was reduced if a high dietary Ca/aP ratio was fed (32.2% vs. 28.9%) compared to birds fed the standard or low Ca/aP diets, whereas in case of a low aP level faecal P digestibility was increased in birds fed the high dietary Ca/aP ratio compared to the other two Ca/aP ratios (44.2 vs. 40.0%).
Phosphate excretion was affected by a GR * sex interaction (Table 17). In standard growing conditions, phosphate excretion was higher in females than males (1.05 vs. 1.01 g/bird/d), whereas phosphate excretion in slow growing conditions was higher in males than females (1.00 vs. 0.91 g/bird/d). Moreover, phosphate excretion was affected by a Ca/aP ratio * GR * aP interaction, as shown in Table 16. In birds fed diets with a high aP level, phosphate excretion of the standard growing birds was consistently higher compared to the slow growing birds in case of a high (1.37 vs. 1.15 g/bird/d), standard (1.22 vs. 1.16 g/bird/d), and low (1.18 vs. 1.13 g/bird/d) Ca/aP ratio. Ca/aP ratio and GR did not affect phosphate excretion in birds fed low aP diets.

Table 15. Interaction effects of Ca/aP ratio, growth rate (GR) and sex on P content of tibia (g/kg).

Treatment	P content tibia (g/kg)
Ca/aP High	
GR Standard	
Male	160.0[bc]
Female	165.2[b]
Ca/aP High	
GR Low	
Male	179.5[a]
Female	166.0[b]
Ca/aP Standard	
GR Standard	
Male	157.2[bc]
Female	166.0[b]
Ca/aP Standard	
GR Low	
Male	157.7[bc]
Female	166.5[b]
Ca/aP Low	
GR Standard	
Male	164.2[b]
Female	153.5[c]
Ca/aP Low	
GR Low	
Male	161.0[bc]
Female	170.0[ab]
SEM	4.80
P-value	**0.032**

3.6 Bird performance

Ca/aP ratio affected BW at 35 d of age (Table 9). BW of birds that were fed the standard or high Ca/aP ratio was 3% higher compared to birds fed the low Ca/aP ratio (1555 vs. 1508 g). Differences in BW between treatments, however, disappeared at d42 ($P = 0.083$) and d49, indicating that the starter and grower period were more sensitive to Ca/aP ratio than the finisher period. BW at d35 was affected by a GR * sex interaction (Table 17). In standard growing birds, sex did not affect BW at d35 (on average 1684 g), whereas BW at d35 of slow growing males was increased compared to slow growing females (1455 vs. 1334 g). Feeding the high aP level resulted in a 34 g increase of BW at d35 (1556 vs. 1522 g).

BW at d42 was increased in standard growing compared to slow growing birds (2333 vs. 1828 g). BW at 42 d of age was affected by an aP * sex interaction, as shown in Table 18. In males, aP level did not affect BW at d42 (on average 1578 g), whereas feeding a low aP level to females reduced BW at d42 (1519 vs. 1482 g).

Feeding a low aP diet to slow growing birds resulted in a reduced BW at 49 d of age (2315 vs. 2212 g). At d49, males were 203 g heavier than females (2365 vs. 2162 g).

Ca/aP ratio and aP level did not affect feed intake level (d10 – d42). Standard growing birds consumed during this period 17.3 g/d more feed compared to the slow growers. Male birds consumed during this period 7.0 g/d more feed compared to the females.

Over the period of 10 to 42 d of age, standard growing birds had a 31% increased daily gain compared to slow growing birds (67.3 vs. 51.5 g/d). Daily gain was affected by an aP level * sex interaction (Table 18). In case of feeding a high aP level, males gained 13% faster compared to females, whereas the difference between sexes was only 6% if a diet with a low aP level was fed.

The dietary Ca/aP ratio did not affect FCR over the period of d10 – d42. As a consequence of restricted feeding of a low protein diet, the FCR of the slow growing birds increased by 10% compared to the standard growing birds (1.77 vs. 1.61). FCR was affected by an aP * sex interaction (Table 18). In case of feeding a high aP level, FCR of males was 5% reduced compared to FCR of females, whereas sex did not affect FCR if a diet with a low aP level was fed.

Table 16. Interaction effects of Ca/aP ratio, growth rate (GR) and aP level on ash content of tibia (g/kg), ileal and faecal Ca digestibility (%) and phosphate excretion (g/bird/d).

Treatment	Ash content tibia (g/kg)	Ileal Ca digestibility (%)	Faecal Ca digestibility (%)	Phosphate excretion (g/bird/d)
Ca/aP High				
GR Standard				
aP High	477^{ab}	20.7^{e}	19.0^{g}	1.37^{a}
aP Low	483^{a}	52.2^{b}	44.2^{d}	0.80^{de}
Ca/aP High				
GR Low				
aP High	448^{d}	13.2^{f}	35.4^{e}	1.15^{c}
aP Low	479^{ab}	39.3^{c}	48.0^{c}	0.76^{e}
Ca/aP Standard				
GR Standard				
aP High	459^{c}	31.5^{d}	36.4^{e}	1.22^{b}
aP Low	482^{a}	50.6^{b}	52.4^{b}	0.83^{d}
Ca/aP Standard				
GR Low				
aP High	450^{d}	23.1^{e}	26.6^{f}	1.16^{c}
aP Low	473^{b}	32.8^{d}	45.7^{d}	0.76^{e}
Ca/aP Low				
GR Standard				
aP High	444^{d}	49.3^{b}	51.1^{b}	1.18^{bc}
aP Low	474^{b}	58.0^{a}	67.9^{a}	0.80^{de}
Ca/aP Low				
GR Low				
aP High	415^{e}	37.9^{c}	48.0^{c}	1.13^{c}
aP Low	442^{d}	58.8^{a}	67.9^{a}	0.79^{de}
SEM	3.9	2.35	1.40	0.026
P-value	**0.029**	**0.008**	**0.007**	**0.041**

Table 17. Interaction between Growth Rate (GR) and sex on phosphate excretion (g/bird/d) and bird weight on d 35 (g).

Treatment	Phosphate excretion (g/bird/d)	Bird weight d35 (g)
GR Standard		
Males	1.01^{b}	1701^{a}
Females	1.05^{a}	1666^{a}
GR Low		
Males	1.00^{b}	1455^{b}
Females	0.91^{c}	1334^{c}
SE	0.015	15.8
P-value	**<.001**	**0.012**

Table 18. Interaction between Growth Rate (GR) and sex on bird weight on d 35 (g), daily gain (g) and FCR over d10 – d42.

Treatment	Bird weight d35 (g)	Daily gain d10 – d42 (g/d)	FCR d10 – d42 (g/g)
aP High			
Males	1593[a]	64.7[a]	1.613[b]
Females	1519[b]	57.1[b]	1.697[a]
aP Low			
Males	1562[a]	59.5[b]	1.731[a]
Females	1482[c]	56.1[c]	1.712[a]
SE	15.8	1.01	0.024
P-value	**0.047**	**0.048**	**0.038**

4 Discussion

4.1 Effect of Ca/aP ratio

Ca and P are stored in bones as hydroxyapatite, $Ca_5(PO_4)_3(OH)$ (Lian et al., 1993). A lot of studies have shown the importance of an appropriate dietary balance between Ca and (available) P (Dieckmann, 2004; Bunzen et al., 2007; Narcy et al., 2009; Delezie et al., 2012).
A high ratio between Ca and P in the diet might increase the risk for a dietary Ca excess. Ca excess was reported to antagonize digestive utilisation of P by decreasing the amount of P in an absorbable form trough the formation of calcium phosphate insoluble precipitates (Ca_2PO_4) (Hurwitz and Bar, 1971). In the current experiment, indeed, a higher Ca/aP ratio significantly reduced the ileal Ca and P digestibility. These findings were confirmed by results of Delezie et al. (2012). From a study, in which the Ca/total P ratio ranged from 1.2 to 3.3, it was concluded that this ratio should not exceed 2.2, because P and Ca digestibility significantly decreased at higher values (Dieckmann, 2004). Comparable Ca/total P ratios are also recommended by NRC (1994) (2.2) and Bunzen et al. (2007) (2.0). Besides negative effects on Ca and P digestibility, Delezie et al. (2012) reported a decreased feed intake and growth rate as a result of supplementing a high dietary Ca/total P ratio, but these findings were not observed in the current experiment. Feeding a high Ca/aP ratio in the current experiment, however, resulted in a low N content of the birds, a moderate reduction in relative skeleton portion (% of EBW), and a reduced relative tibia weight (% of EBW), compared to the standard Ca/aP ratio, indicating that the amount of available P became limiting for optimal performance of the high Ca/aP fed birds. Feeding the highest Ca/aP ratio, however, showed the highest tibia strength and Ca and P contents in the tibia.
On the other hand, Dieckman (2004) found no effect of a low Ca/total P ratio on P and Ca digestibility, and therefore no minimum ratio could be derived from that study. Despite the high Ca and P digestibility, a low dietary Ca/aP ratio in the current experiment reduced the P content of the birds, the relative portion of skeleton (% of EBW), the ash and Ca content in the skeleton, the relative tibia weight, the tibia strength and the daily gain in the starter and grower period of the birds, compared to the standard Ca/aP ratio. All these results are indicating that the Ca supply in case of the low Ca/aP ratio did not fully support the requirement of the birds. Létourneau-Montminy et al. (2010) suggested that the Ca/total P ratio should be reduced if broilers are fed diets with a low non-phytate P content to ensure maximum response on performance. It was hypothesized that a low Ca/aP ratio was more critical for bone development in case of a marginal aP supply. Although not significant ($P = 0.114$), in the present experiment the portion of P in the skeleton tended to be reduced if birds were fed a diet with a low aP content and a low Ca/aP ratio (46.4% versus 53.1-58.8% in the other treatment combinations). Likewise, the tibia ash content was reduced ($P = 0.003$) if birds were fed a diet with a low aP content and a low Ca/aP ratio (428 g/kg versus 459-480 g/kg in the other treatment combinations). These findings confirmed our hypothesis. In the present experiment, performance traits were not affected by interactions between Ca/aP ratio and aP level, showing that the Ca and P supply is more sensitive for bone development than for performance traits.
It can be concluded that applying the standard Ca/aP ratio (2.2) was beneficial for bone development and performance characteristics compared to the findings with the low (1.5) and the high (2.9) Ca/aP ratio.

4.2 Effect of aP level

P is an essential nutrient in broiler diets, which is closely involved in bone mineralisation, muscle development, metabolic processes and growth (Kornegay et al., 1996; Park et al., 2009). In the present experiment, feeding the low aP grower diet (2.4 g aP/kg) resulted in a 5% reduction in daily gain and in a 4% increase in FCR compared to feeding the high aP grower diet (3.6 g aP/kg). In a dose – response study, in which total P contents of grower diets ranged from 5.0 to 6.5 g/kg, corresponding with 2.9 to 3.9 g/kg non-phytate P (nPP) the P content, however, did not significantly affect BWG, FCR and breast muscle yield (Abudabos, 2012). Based on several experiments, in which total dietary P content ranged from 2 to 10 g/kg, feed intake, daily gain, and FCR were not affected by P supply (Dieckmann, 2004). In contrast with these findings, the results of the current experiment showed that an aP level of 2.4 g/kg in the grower phase did not fully support the growth potential of broilers. Also results of Létourneau-Montminy et al. (2010), which are based on a meta-analysis of

results published in literature, confirmed that performance parameters were dependent on the nPP supply. These authors provided exponential equations that fit the relation between the dietary nPP content and performance parameters. The response of the nPP content, however, depended on the dietary Ca level. A nPP level of 2.9 g/kg, corresponding with 5 g/kg total P, in the study of Abudabos (2012) seemed to be sufficient for maximal growth performance and skeletal integrity, whereas Létoureau-Montminy et al. (2010) estimated a nPP requirement of 4.0 g/kg (Ca level of 6 g/kg) to 4.4 g/kg (Ca level of 10 g/kg) for realising maximal BWG in broilers at 21 d of age. The reduction of BWG in case of feeding a P-deficient diet could be the result of a loss of appetite, consequently reducing feed intake (Narcy et al., 2009; Letourneau-Montminy et al., 2010). Moreover, P deficiency might negatively affect important processes in body metabolism related to e.g. formation of nucleic acids and various enzymatic reactions (Kornegay et al., 1996).
In the present experiment, Ca and P contents of the soft tissues were not affected by Ca/aP ratio and aP level, indicating that birds gave preference to fulfil the requirements of these minerals in soft tissue, rather than in bones.
In the study of Abudabos (2012), an increase of the nPP content of the diet from 2.4 to 4.0 g/kg resulted in a large increase of the tibia ash content (from 396 g/kg to 444 g/kg). In the present experiment, birds fed the low aP diets already had a relative high tibia ash content (450 g/kg), and this content increased to 459 g/kg if the birds were fed the high aP diets. These findings indicated that the aP requirement of the birds in the current experiment were already close to the requirement at the low aP level, whereas the birds in the experiment of Abudabos (2012) showed a much stronger response of increasing nPP levels on tibia ash content.
In the current experiment, phosphate excretion was only marginally affected by Ca/aP ratio and GR in birds fed the low aP level (ranging from 0.76 to 0.83 g/d), whereas in standard growing birds fed the high aP level phosphate excretion significantly increased if the high Ca/aP ratio was applied compared to the other Ca/aP ratios (1.37 vs. 1.20 g/d). Abudabos (2012) determined a linear relationship between dietary P content, P intake and P excretion. In line with our study, Abudabos (2012) reported that the Ca and P digestibility decreased with increasing nPP levels.
Besides the negative effects on performance, supplying the low aP level in the current experiment resulted in a reduced P content in EBW, relative skeleton portion, ash content in skeleton, and P content in skeleton. The low aP level did not affect tibia characteristics, except tibia ash, but improved ileal P digestibility and reduced P excretion. Similar effects of P digestibility and excretion were also reported by other authors (Manangi and Coon, 2008; Delezie et al., 2012).
In the current experiment, tibia ash was slightly increased by feeding the high aP level. According to Narcy et al. (2009) the maximisation of tibia mineralisation requires a higher dietary aP than those optimising growth performance criteria.
This experiment showed that feeding broilers the high aP level, both the performance level as well as the level of bone mineralisation increased. Therefore, it can be concluded that the low dietary aP level did not fully cover the P requirement of the birds. Further research, using dose – response studies, however, is necessary to determine the precise P requirement of broilers. Such a study should be performed under the condition of a standard Ca/aP ratio.

4.3 Effect of growth rate (GR)

Modern broilers are selected for a high daily gain, especially to produce a lot of meat (Havenstein et al., 2003). It was hypothesized that the development of the skeleton in fast-growing broilers could not keep pace with the gain of the soft tissues. Indicators of a less well developed skeleton could be among others i) a low relative skeleton weight (% of EBW), ii) a low Ca and P content in the skeleton, iii) a reduced bone strength, and iv) increased locomotion problems. To test this hypothesis, the factor GR was included in this experiment. In line with our hypothesis it was observed that standard growing birds had a reduced relative skeleton weight (8.2 vs. 7.1% of EBW) and relative tibia weight (1.13 vs. 1.01% of EBW) compared to the slow growing birds. The Ca and P contents of the total skeleton, however, were not affected by GR, although the tibia of the slow growing birds contained more Ca and P. Despite the lower tibia ash content, the breaking strength of the tibia of standard growing birds was even increased compared to the slow growing broilers. The gait score determined on d28 was increased in the standard growing broilers compared to the slow growing broilers, indicating an increase in locomotion problems. The differences in gait score on that day, however, were confounded by different BW of both groups, and therefore this difference cannot be exclusively attributed to the GR effect, as stated by Waldenstedt (2006).

Our results are in line with those of Erdal et al. (2012), who also observed that the bone strength of slow growing broilers, which were fed restrictedly, was reduced compared to birds fed a control diet *ad libitum*. Reducing the GR of broilers by providing a low energy diet, however, did not reduce the bone strength of the birds (Erdal et al., 2012). These authors suggested that the nutrient supply of the restricted-fed birds was inadequate for realizing an optimal bone development.
Based on the results of this experiment, it can be concluded that there is no sound proof for a retarded skeleton development in standard growing broilers compared to slow growing broilers. It was hypothesized that the aP requirement of fast growing broilers might be underestimated because of a this suggested retarded skeleton development, but this could not be confirmed by our findings.

4.4 Dietary aP-requirement according to factorial approach

The P content/kg EWB in the current study was affected by Ca/aP ratio, aP level and sex, but amounted among treatments 4.6 g/kg EBW (male 4.7 g/kg EBW, female 4.5 g/kg EBW). Dieckmann (2004) reported an average P content of broilers of 4.8 g/kg, also with higher values for male birds compared to females (5.0 vs. 4.6 g/kg), while comparable values were reported by WPSA (1985), ranging from 4.9 g/kg at 3 wk of age to 4.7 g/kg at 8 wk of age. In contrast, lower P contents at slaughter were reported by Van der Klis and Blok (1997) (4.0 g/kg) and Versteegh and Jongbloed (2000) (4.4 g/kg). In Figure 3, the relation between age and P content in broilers, as presented by different authors, are shown. The values of the current experiment are based on the average values of all the treatments.

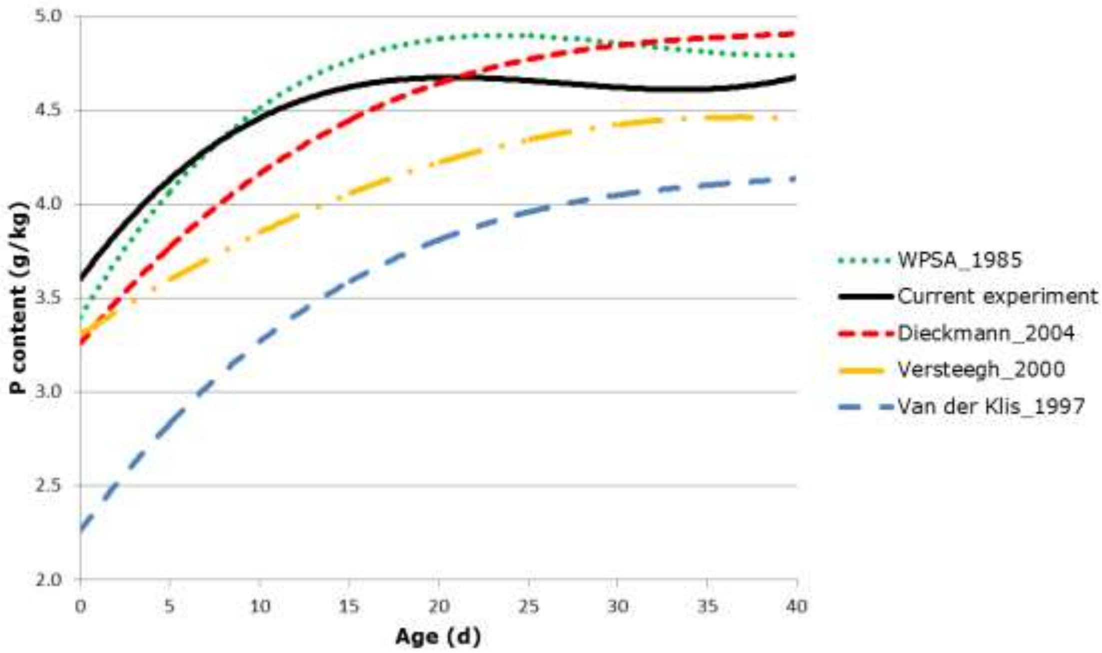

Figure 3. The relation between age and P content, as presented by different authors.

Based on the feed intake and BW levels provided by Ross (2012), and on the P contents of the broilers in the current experiment, the aP requirement in broilers is calculated according to the factorial approach system that is common in the Netherlands (Van der Klis and Blok, 1997). Starter diets (0 – 10 d of age) should contain an aP level of 5.5 g/kg, grower diets (10 – 30 d of age) 3.3 g/kg and finisher diets (> 30 d of age) 2.9 g/kg. These requirements are 37%, 7% and 4% higher compared to the current Dutch recommendations (CVB, 2012), respectively, indicating that there is an urgent need for updating the aP requirement of starter diets.

4.5 Ileal versus faecal Ca and P digestibility

Among all treatments, faecal Ca digestibility is 4% higher compared to the ileal Ca digestibility level (43 vs. 39%), indicating that a small amount of Ca is further digested in the hindgut. Faecal P digestibility coefficients, however, were 10.5% lower compared to ileal P digestibility (46.8 vs. 36.3%). Part of the ileal absorbed P is excreted by the urine into the faeces, which explains the reduced faecal digestibility coefficients. Therefore, ileal digestibility values provide a better insight in the P metabolism compared to faecal digestibility.

5 Conclusions

- The contents of ash, Ca and P in soft tissues were not affected by aP level and Ca/aP ratio. The P and N content in soft tissues of slow growing birds were reduced compared to standard growing birds.
- Among treatments, 75.3% of body Ca and 53.6% of body P was deposited in the skeleton.
- Among treatments, P and N contents in empty body weight amounted 4.6 and 29.7 g/kg, respectively.
- Broilers that are fed a high dietary aP level (20% higher than recommended) had a 8% higher P content in empty body weight, because of an increased skeleton portion and an 8% elevated P content in the skeleton, compared to broilers fed a low dietary aP level (20% below the recommended level). Feeding the diet with the high aP level resulted in improved BWG (5%) and FCR (4%). Feeding a high dietary aP level reduced ileal Ca and P digestibility, whereas faecal phosphate excretion increased by 53%.
- A low Ca/aP ratio (1.5) in the diet reduced BWG in the starter and grower period, and the P content in empty body weight, compared to a standard (2.2) or high (2.9) Ca/aP ratio. In birds fed a high dietary aP level, a high Ca/aP ratio resulted in an increased phosphate excretion compared to birds fed the standard or low Ca/aP ratio. The ash content in the skeleton, and the tibia strength increased with increasing Ca/aP ratio, whereas the ileal Ca and P digestibility linearly decreased with an increasing Ca/aP ratio. Applying the standard Ca/aP ratio resulted in the highest N content in soft tissue and in empty body weight, in the highest skeleton portion and Ca and P content in the skeleton, in the highest (relative) tibia weight, and in the highest BW at 35 d of age.
- The P content in slow growing birds did not significantly differ from that of fast growing birds. The skeleton portion in empty body weight was increased by 15% in slow growers compared to fast growers, consequently resulting in an elevated Ca and P portion in the skeleton. The tibia of slow growers contained more Ca and P, but the tibia strength was reduced compared to the fast growers.

<u>Recommendations</u>
Feeding a diet with a low Ca/aP ratio (1.5) negatively affected broiler performance, whereas the Ca and P digestibility reduced with increasing dietary Ca/aP ratios. In line with our hypothesis, it was observed that feeding a diet with the standard Ca/aP ratio (2.2) resulted in a similar or better skeleton and tibia development compared to the high Ca/aP ratio (2.9). Therefore, the standard Ca/aP ratio can be recommended for application in practical diets. Based on the results of this experiment, no proof was found for our hypothesis that the development of the skeleton in fast growing broilers could not keep pace with the gain of the soft tissues.
According to the factorial approach system that is common in the Netherlands, starter diets (0 – 10 d of age) should contain an aP level of 5.5 g/kg, grower diets (10 – 30 d of age) 3.3 g/kg and finisher diets (> 30 d of age) 2.9 g/kg. These requirements are 37%, 7% and 4% higher compared to the current Dutch CVB recommendations.
A new animal experiment has to be performed to determine whether the results of the factorial approach are in line with the findings measured in the birds itself. This experiment showed that the P requirement was not fulfilled with the low aP level in the diet. For determination of the optimal dietary aP level, however, a dose – response experiment should be performed. This additional study will be carried out in 2013/2014.

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